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JOURNAL

OF THE

FISHERIES RESEARCH BOARD OF CANADA

(Formerly the Biological Board of Canada)

Volume VII

1947-1950

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Printed by
THE UNIVERSITY OF TORONTO PRESS
for the
FISHERIES RESEARCH BOARD OF CANADA

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Water Transports and Current Patterns for the Scotian Shelf

By H. B. Hachey
Atlantic Biological Station
Received for publication November 8, 1945

ABSTRACT

Data of twelve seasonal cruises, during 1934-1938, over the Scotian shelf have been used to determine surface currents, volume transports, and current patterns. Surface currents ranged from zero to 37.9 cm./sec. (18.2 nautical mi. da.). Volume transports varied considerably from season to season, and were greatest during winter months. Isentropic analysis of the data indicated a persistent current pattern, defining a water movement parallel to the coast, a large-scale circulatory movement over the central portion of the shelf, and a complicated vortex in the southwesterly portion of the area.

INTRODUCTION

During the last fifteen years or more, the emphasis in physical oceanography has been on deep water circulation. Few attempts have been made to apply modern theory to the more complicated situations encountered in shallow areas. Until better techniques are developed for observing ocean currents directly, the usefulness of the theory must, to a considerable extent, be judged by the consistency of the results obtained when it is applied to routine temperature and salinity observations. It will be a long time before physical oceanographers can work with entirely satisfactory basic data. Synoptic observations are practically unobtainable and very large interpolations are customarily made between individual readings.

The present study is a preliminary attempt to apply some of the methods, used in deep water, to a series of temperature and salinity observations from over the continental shelf. While the results are generally consistent, only future observations of the actual movement of the water will show to what extent the calculated values correspond with reality.

Collection of Data

During the years 1934 to 1938 inclusive, the Fisheries Research Board of Canada was engaged in oceanographical investigations on the Scotian shelf (Hachey 1937), an area which forms a transition zone between coastal and oceanic waters. From the hydrographical point of view, physical and chemical conditions of the waters in this area are determined in the main by water contributions from two sources, the water area to the east and north, and the water area to the south. To the east and north is a limitless supply of waters of arctic origin, while two to four hundred miles (1 mi. equals 1.6 km.) to the south is the Gulf Stream system, whose waters originate in tropical latitudes. The interpre-

J. Fish. Res. Bd. Can. 7 (1) 1947.
Printed in Canada.

TABLE I. Volume transport in upper thirty meteres—cu.m./sec.

Section	121-124				58-50		
Date	121-122	122-123	123-124	121-124	58-126	126-50	58-50
1934							
	N.E.	N.E.	S.W.	S.W.	S.W.	S.W.	S.W.
May	15,500	11,000	167,400	140,900	53,300	9,300	62,600
	N.E.	S.W.	S.W.	S.W.	S.W.	N.E.	S.W.
Aug.	76,300	45,400	31,700	800	47,800	9,300	38,500
1935							
	N.E.	N.E.	S.W.	S.W.	S.W.	S.W.	S.W.
May	1,700	900	24,200	21,600	52,300	113,000	165,300
	N.E.	S.W.	N.E.	N.E.	N.E.	S.W.	N.E.
Aug.	40,500	7,900	2,100	34,700	63,000	46,100	16,900
1936							
	S.W.	N.E.	S.W.	N.E.	S.W.	S.W.	S.W.
Feb.	28,000	43,000	11,300	3,700	43,200	178,000	221,200
	N.E.	S.W.	N.E.	N.E.	S.W.	S.W.	S.W.
May	22,600	4,400	18,800	37,000	8,100	11,600	19,700
	S.W.	S.W.	N.E.	N.E.	S.W.	S.W.	S.W.
Aug.	15,600	11,500	30,400	3,300	17,400	8,100	25,500
1937							
	S.W.	S.W.	S.W.	S.W.	S.W.	S.W.	S.W.
Feb.	25,600	70,400	77,700	173,700	125,500	97,800	223,300
	N.E.	S.W.	N.E.	S.W.	S.W.	S.W.	S.W.
May	2,700	47,300	18,600	26,000	21,600	112,000	133,600
	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Aug.	39,600	38,600	2,300	80,500	118,400	2,500	120,900
1938							
	S.W.	N.E.	S.W.	S.W.	S.W.	S.W.	S.W.
Jan.	1,100	10,900	79,900	70,100	215,000	9,700	224,700
	S.W.	N.E.	N.E.	S.W.	S.W.	S.W.	S.W.
Ap.-May	18,500	8,000	1,100	9,400	79,700	83,700	163,400
	N.E.	S.W.	S.W.	S.W.	S.W.	S.W.	S.W.
Au.-Sep.	13,400	19,800	33,900	40,300	38,800	42,800	81,600
	Surface current in cms./second						
1934							
May	1.9 N.E.	2.7 N.E.	23.2 S.W.	5.1 S.W.	5.0 S.W.	0.4 S.W.	2.3 S.W.
Aug.	11.3 N.E.	4.2 S.W.	3.2 S.W.	2.1 N.E.	1.2 S.W.	1.0 N.E.	0.1 N.E.
1935							
May	0.1 S.W.	0.0	3.4 S.W.	1.0 S.W.	7.0 S.W.	8.7 S.W.	8.0 S.W.
Aug.	8.7 N.E.	0.4 S.W.	0.6 S.W.	3.1 N.E.	10.3 N.E.	4.6 S.W.	1.5 N.E.
1936							
Feb.	3.6 S.W.	5.5 N.E.	1.4 S.W.	0.1 S.W.	4.5 S.W.	14.5 S.W.	10.4 S.W.
May	3.3 N.E.	0.3 S.W.	3.4 N.E.	2.3 N.E.	0.6 S.W.	0.8 S.W.	0.7 S.W.
Aug.	2.0 N.E.	1.5 S.W.	0.5 N.E.	1.8 N.E.	2.1 S.W.	0.1 N.E.	0.8 S.W.
1937							
Feb.	2.8 S.W.	10.0 S.W.	12.3 S.W.	7.9 S.W.	14.7 S.W.	7.3 S.W.	10.3 S.W.
May	0.5 S.W.	6.3 S.W.	37.9 S.W.	13.3 S.W.	3.6 S.W.	9.1 S.W.	6.9 S.W.
Aug.	7.6 N.E.	9.7 N.E.	0.4 N.E.	6.3 N.E.	22.8 N.E.	0.1 S.W.	9.2 N.E.
1938							
Jan.	0.7 S.W.	11.3 S.W.	1.6 N.E.	3.5 S.W.	24.8 S.W.	0.7 S.W.	10.5 S.W.
Ap.-Ma.	2.0 S.W.	1.5 N.E.	0.7 N.E.	0.1 S.W.	9.7 S.W.	5.7 S.W.	7.3 S.W.
Au.-Sep.	3.8 N.E.	0.9 S.W.	4.5 S.W.	0.1 S.W.	2.2 S.W.	3.4 S.W.	2.9 S.W.

TABLE I. Volume transport in upper thirty meters—cu.m/sec.

Section	127-129			134-131			
Date	127-128	128-129	127-129	134-133	133-132	132-131	134-131
1934							
May	S.W. 133,000	N.E. 17,300	S.W. 115,700	S.W. 50,700	S.W. 15,400	N.E. 19,300	S.W. 46,800
Aug.	N.E. 17,200	N.E. 84,400	N.E. 101,600	S.W. 8,200	S.W. 9,200	S.W. 50,200	S.W. 67,600
1935							
May	S.W. 129,000	S.W. 16,800	S.W. 145,800	S.W. 56,000	N.E. 900	N.E. 24,200	S.W. 30,900
Aug.	S.W. 9,600	N.E. 40,400	N.E. 30,800	S.W. 24,600	N.E. 17,400	N.E. 6,200	S.W. 1,000
1936							
Feb.	S.W.	N.E.	S.W.	S.W.	S.W.	N.E.	S.W.
May	71,800	24,400	47,400	62,800	16,700	30,600	48,900
Aug.	S.W. 99,100	N.E. 109,000	N.E. 9,900	S.W. 134,000	N.E. 13,200	N.E. 15,700	S.W. 105,100
1937							
Feb.	S.W. 133,000	N.E. 116,000	S.W. 17,000	S.W. 34,100	S.W. 10,200	N.E. 36,400	S.W. 7,900
May	S.W. 68,300	N.E. 13,200	S.W. 55,100	N.E. 21,400	S.W. 40,600	N.E. 1,600	S.W. 17,600
Aug.	S.W. 76,200	N.E. 90,000	N.E. 13,800	S.W. 86,800	N.E. 5,660	N.E. 45,800	S.W. 35,340
1938							
Jan.	S.W. 304,000	N.E. 62,300	S.W. 231,700	S.W. 113,000	S.W. 19,700	N.E. 7,900	S.W. 124,800
Ap.-May	S.W. 138.000	S.W. 17,600	S.W. 155,600	S.W. 72,000	N.E. 4,600	N.E. 65,200	S.W. 2,200
Au.-Sep.	S.W. 55,200	S.W. 1,400	S.W. 56,600	N.E. 24,600	S.W. 26,800	N.E. 15,300	N.E. 13,100
	Surface current in cms./second						
1934							
May	10.3 S.W.	0.9 N.E.	3.8 S.W.	5.3 S.W.	2.2 S.W.	1.1 N.E.	2.3 S.W.
Aug.	2.4 N.E.	4.4 N.E.	3.5 N.E.	2.5 N.E.	2.3 S.W.	7.4 S.W.	2.2 S.W.
1935							
May	12.1 S.W.	1.1 S.W.	5.6 S.W.	6.3 S.W.	1.7 S.W.	1.5 N.E.	1.5 S.W.
Aug.	1.1 N.E.	1.4 N.E.	1.3 N.E.	1.8 S.W.	2.5 N.E.	2.1 S.W.	0.6 S.W.
1936							
May	5.9 S.W.	1.2 N.E.	1.8 S.W.	6.0 S.W.	1.8 S.W.	2.5 N.E.	1.9 S.W.
Aug.	9.0 S.W.	6.2 N.E.	0.2 S.W.	12.7 S.W.	0.9 S.W.	2.5 S.W.	5.8 S.W.
1937							
Feb.	11.5 S.W.	6.3 N.E.	1.2 S.W.	3.5 S.W.	0.9 S.W.	4.4 N.E.	0.1 S.W.
May	7.2 S.W.	0.6 N.E.	2.5 S.W.	1.1 N.E.	6.3 S.W.	0.2 S.W.	1.6 S.W.
Aug.	10.9 S.W.	5.2 N.E.	1.6 S.W.	10.7 S.W.	0.8 N.E.	2.9 N.E.	2.6 S.W.
1938							
Jan.	23.9 S.W.	2.7 N.E.	8.5 S.W.	10.5 S.W.	1.5 S.W.	0.4 N.E.	4.2 S.W.
Ap.-Ma.	11.8 S.W.	1.2 S.W.	5.6 S.W.	7.2 S.W.	0.9 N.E.	6.3 N.E.	0.3 S.W.
Au.-Sep.	4.5 S.W.	0.2 S.W.	2.0 S.W.	0.4 N.E.	3.4 S.W.	0.1 S.W.	0.9 S.W.

tation of the general hydrographic conditions experienced on the Scotian shelf depends on a proper assessment of the individual seasonal contributions from these two sources.

Twelve seasonal hydrographic cruises were made in the period 1934 to 1938 over a network of stations on the Scotian shelf (figure 1). The network consisted of sixteen stations, which were occupied during each spring and summer. Complete winter cruises were made in 1937 and 1938, while several stations were also occupied in the winter of 1936 (Hachey 1938). A cruise of the area was generally completed in five to ten days.

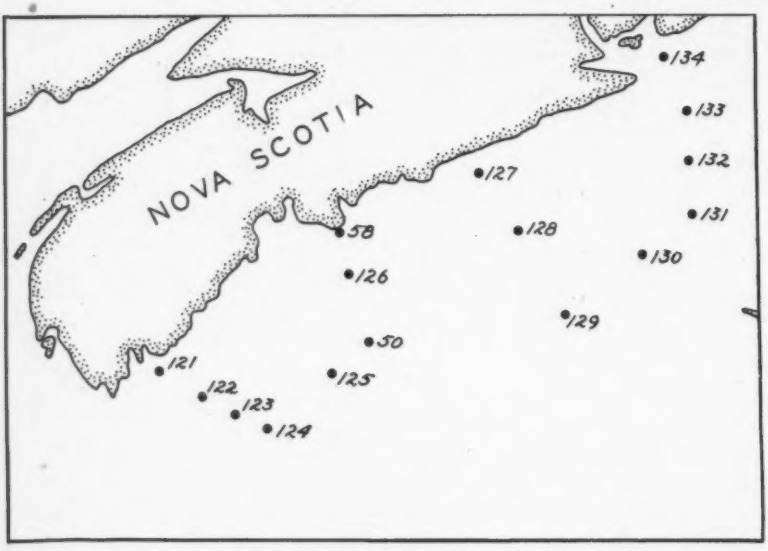

FIGURE 1. Location of hydrographic stations on the Scotian shelf.

The general procedure at a station was to obtain a series of water temperatures at regular depth intervals between surface and bottom. At the same time, water samples were obtained for salinity determinations. With these basic data (Hachey unpub.), seasonal water transports and current patterns have been determined for the Scotian shelf.

WATER TRANSPORTS

In a previous publication (Hachey 1938) the method of calculating volume transports and surface velocities from the basic data of temperature and salinities has been outlined. The same methods, used herein, furnish the detailed volume transports and surface currents of table I. The network of stations of figure 1 resolves itself into four sections: 121-124, 58-50, 127-129, and 134-131, all of which are roughly at right angles to the Nova Scotia coast.

Volume Transports of the Upper Thirty Metres

The volume transports at right angles to the sections, approximately northeast or southwest, for the upper thirty metres of water, are furnished in some detail in table I, expressed in cu.m./sec.

The winter volume transport through the upper thirty metres of section 121-124 was towards the northeast in 1936, when the northeast transport in February, between stations 122-123, of 43,000 cu.m./sec. more than offset the southwest transports through other portions of the section. In 1937 and 1938, the southwest winter transport through this same section was 173,700 and 70,100 cu.m./sec. respectively. The May transports through section 121-124 were towards the southwest, except in 1936. Generally, the May transport was towards the northeast between the inshore stations 121 and 122. Generally too, the May transport was towards the southwest between stations 122 and 123 when it was towards the northeast between stations 123 and 124, and vice versa.

The volume transport through the upper thirty metres of section 58-50 was, in the winter months, as much as 224,700 cu.m./sec. towards the southwest. While a regular southwest transport is generally a feature of the section at all seasons, the winter transport far surpasses that of other seasons. The transport through this section generally decreases in volume through spring and summer and may be, as in August 1935 and in August 1937, towards the northeast.

The winter transport through the upper thirty metres of section 127-129 is generally towards the southwest through the inshore portion of the section, and towards the northeast between the offshore stations 128 and 129. The winter transport between stations 127 and 128 in the upper thirty metres was more than 300,000 cu.m./sec. in 1938. Generally, the southwest transport between stations 127 and 128 decreases through spring and summer, while the northeast transport between stations 128 and 129 increases. The resultant transport, through the upper thirty metres of the section, is generally towards the northeast in August.

The resultant volume transport in the upper thirty metres of the section 134-131 is towards the southwest, except during the summer of 1938. The resultant spring transport of 30,900 cu.m./sec. to the southwest in 1935, the resultant summer transport of 35,340 cu.m./sec. to the southwest in 1937, and the resultant winter transport of 124,800 cu.m./sec. to the southwest in 1938 illustrate that the larger volume transports through this section 134-131 may be experienced at any season.

Large Scale Differential Movements

The calculations recorded in table II, which deal with the volume transports for the section 58-50 between the surfaces of 30 and 75 m., indicate that the volume transports are of equivalent magnitude to those of the 0-30 m. layer. In particular, this is true for the whole section 58-50, except in 1937, when the volume transport in August was 120,900 cu.m./sec. to the northeast in the 0-30 m. layer, and 2,900 cu.m./sec. to the northeast in the 30-75 m. layer. This result is indicative of differential movements, i.e. independent movement of two waters

of contrasting physical characteristics. This suggested differential movement may be particularly noted between stations 58-126, where the August transport in 1937 was 118,400 cu.m./sec. to the northeast in the upper thirty metres, and 30,500 cu.m./sec. to the southwest in the layer between the 30 and 75 m. levels.

TABLE II. Volume transport in cu.m./sec., 30-75 m. layer.

	Volume transport cu.m./sec. 30-75 m. layer		
Date	126-50	58-126	58-50
1934			
May	S.W. 18,400	S.W. 72,700	S.W. 91,100
Aug.	S.W. 12,800	—	—
1935			
May	S.W. 135,000	S.W. 65,700	S.W. 200,700
Aug.	S.W. 2,600	N.E. 15,600	N.E. 13,000
1936			
Feb.	S.W. 193,000	S.W. 60,100	S.W. 253,100
May	S.W. 36,000	S.W. 32,500	S.W. 68,500
Aug.	S.W. 4,600	S.W. 39,400	S.W. 44,000
1937			
Feb.	S.W. 128,200	S.W. 145,500	S.W. 275,700
May	S.W. 102,000	N.E. 6,100	S.W. 95,900
Aug.	N.E. 33,400	S.W. 30,500	N.E. 2,900
1938			
Jan.	S.W. 13,500	S.W. 223,000	S.W. 236,500
Ap.-May	S.W. 94,300	S.W. 94,300	S.W. 188,600
Au.-Sep.	S.W. 95,200	S.W. 95,200	S.W. 124,000

SURFACE CURRENTS

The surface currents, expressed in cm./sec. are furnished in table I. The values refer to the current components at right angles to a line joining the par-

ticular pair of stations referred to in the column heading. In particular, it may be said that the persistent surface currents during the winter and spring months are towards the southwest, corresponding in general to the trend of volume transports in the upper thirty metres. In August, the surface currents tend towards the northeast, particularly in the central portion of the shelf. In magnitude. these calculated surface currents have ranged from 0.0 cm./sec. between stations 122-123 in May, 1935, to 37.9 cm./sec. (18.2 nautical miles/day) between stations 123 and 124 in May, 1937. The stronger currents do not persist as such, and have no particular relation to season. A surface current of 23.9 cm./sec. towards the southwest was observed in January 1938, in section 127-129, a surface current of 37.9 cm./sec. towards the southwest in May 1937, in section 121-124, while a surface current of 22.8 cm./sec. towards the northeast was observed in section 58-50 in August 1937.

CURRENT PATTERNS

While the calculated volume transports and surface currents furnish estimates of the magnitude of the water movements on the Scotian shelf, they are not sufficient in themselves in furnishing the actual pattern of the water particles in motion. These current patterns may be deduced from the temperature and salinity observations by charting one of these properties for surfaces of constant potential density. Where pressures do not exceed 1,000 decibars, a surface of constant potential density is practically equivalent to a surface of constant σ_t (a σ_t-surface). This is the general procedure of isentropic analysis as appplied to problems of oceanic circulation (Montgomery 1938) and, in effect, is a method of determining the paths of free water particles. Essentially, the method is based on the fact that a free water particle is constrained to move within and with its proper isentropic surface (in practice a σ_t-surface). If, for example, the distribution of salinity within a given σ_t-surface is charted, the isohalines form a current pattern illustrative of the paths of particles in motion within the given surface. To be of significance, the pattern must have some stability, in that it must represent more than a temporary situation. This stability is dependent upon the rate of change in the physical nature of the water particles considered. Hence, in coastal areas particularly, the significance of the current patterns obtained through isentropic analysis is limited by variation of stability with time.

Seasonal σ_t-Surfaces on the Scotian Shelf

Of the twelve seasonal cruises referred to above, five are illustrative of conditions in spring (later April or May), five of conditions in summer (late August or September), and two of conditions in winter (late January or February). From the temperature and salinity data, corresponding σ_t values were readily calculated (Knudsen 1901), and figures 2 and 3 are illustrative of winter distribution of σ_t in section, in 1937 and 1938, while figures 4 and 5 are illustrative of spring and summer distributions respectively during 1938.

The comparatively unstable nature of the upper water layers during the winter months is illustrated in figures 2 and 3, by the comparative lack of σ_t-stratification, and further by the skewness of the σ_t-surfaces. Such winter features,

extending over the whole network of stations in 1937, are confined chiefly to the coastal areas in 1938, and are brought about chiefly through volume transport of water and not through local convectional processes (Hachey 1938). In table I we find, for the winter of 1938 that much the greater volume transports are confined to coastal areas, whereas in 1937, the volume transports were more or less evenly divided as between offshore and inshore areas. The winter transport

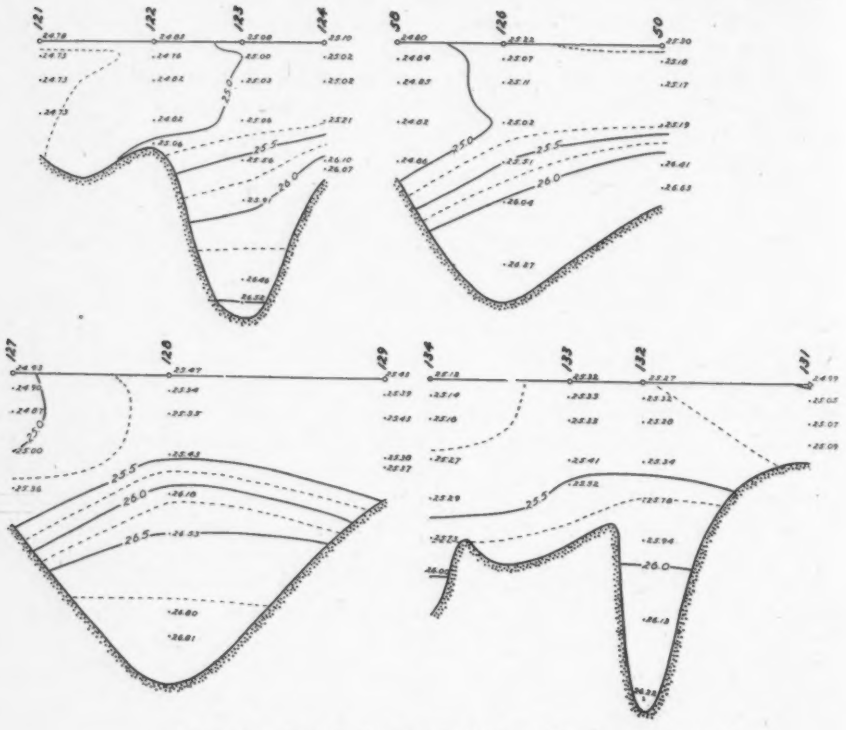

FIGURE 2. Distribution of σ_t in section, February, 1937

from the northeast is generally the greatest transport of the year. As spring approaches, warmer surface waters are responsible for increased stability, and the σ_t-surfaces, in the upper layers, as shown in figure 4 for April, 1938, tend more toward the horizontal. The summer stratification, in the upper layers, chiefly the result of a vertical temperature gradient, is well illustrated in figure 5 for August, 1938, by the number of σ_t-surfaces between depths of 10 and 25 metres.

For purposes of isentropic analysis, it is essential that we choose a σ_t-surface in the upper layers of these sections, which would extend over the whole area investigated. It is also essential that vertical mixing and radiation should be of little significance, in comparison with lateral mixing (Montgomery 1938), within the period of the survey. To conform with these essentials, the methods of

analysis are applied only to the data of the spring and summer cruises for each year of the period 1934-38 inclusive.

ISENTROPIC ANALYSIS OF THE DATA

A σ_t-surface was chosen which would extend throughout the area of the investigation. The salinity associated with this σ_t-surface was determined for

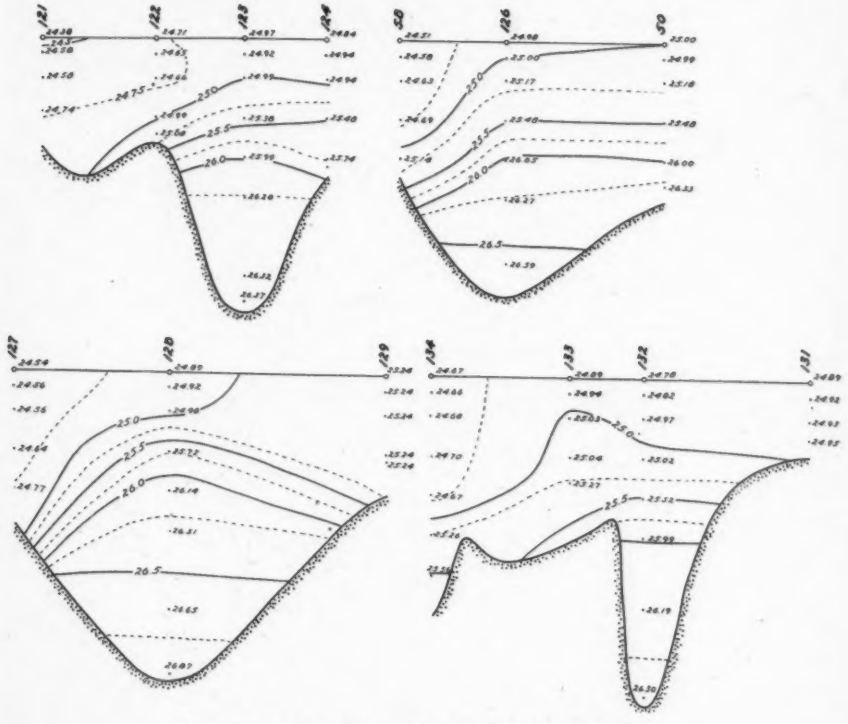

FIGURE 3. Distribution of σ_t in section, January, 1938.

each station of the network (Montgomery 1938). These salinities were then recorded in the charted σ_t-surface and the isohalines drawn. The depths corresponding to the interpolated salinities were obtained from salinity-depth curves, and the general configuration of the σ_t-surface determined. This procedure was followed with respect to the data of five spring and summer cruises of the Scotian shelf, and the charted results are shown in figures 6 and 7.

The current pattern, indicated by arrows, is based upon the following principles:

(*a*) water particles of a given salinity, in the σ_t-surface, are constrained to move along the isohalines;

(*b*) the configuration of the σ_t-surface, as determined by its depth at the

various stations, indicates the direction of flow, a tendency for cyclonic circulation about points where the σ_t-surface is shallow (S), and for anticyclonic circulation about points where the σ_t-surface is deep (D) (R. is used to indicate a ridge between two "deeps").

In charting the distribution of salinity in May (figure 6), the σ_t-surface of 25.50 was chosen, and a definite current pattern is indicated persisting through-

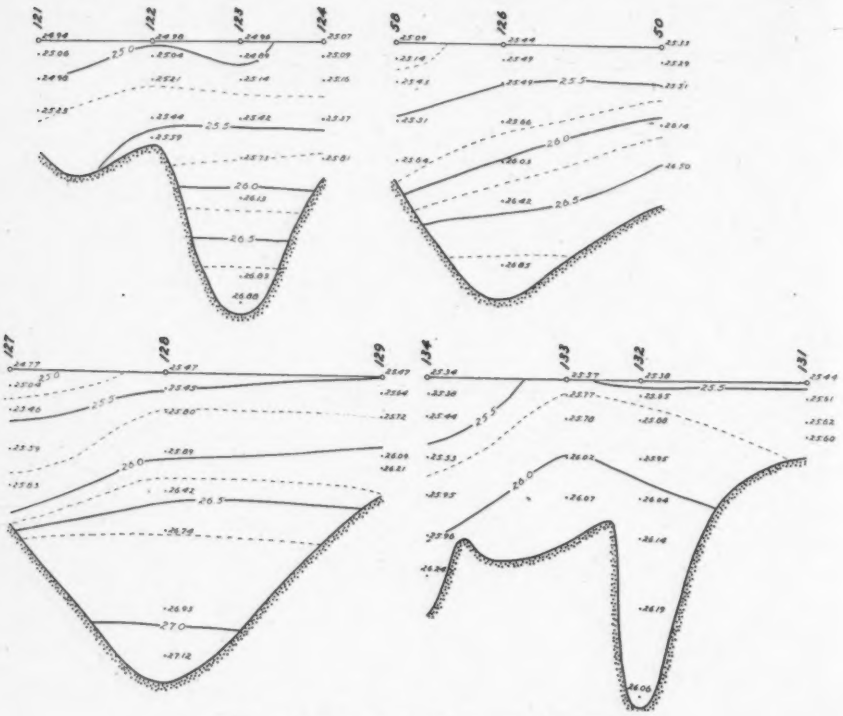

FIGURE 4. Distribution of σ_t in section, April, 1938.

out the five-year period. Probably the most outstanding feature of the pattern is that indicating water moving southwesterly along the coast. There is also evidence of a large scale circulatory movement of the waters over the central portion of the shelf, which in May, 1934, seems to be of particular significance because of the elimination of the coastwise movement. In the southwesterly portion of the area, there is evidence of a complicated vortex, which is particularly well developed in May, 1936.

The distribution of salinity in August (figure 7) is charted in the σ_t-surface of 24.50. The circulatory movement of the waters evident in May over the central portion of the shelf is much more pronounced, except in 1936, when the coastwise movement predominates. The vortex in the southwesterly portion of

the area is much in evidence, except in August, 1938. An outstanding feature is the total disappearance of the southwesterly movement along the coast in 1934.

These charts indicate that there is, on the Scotian shelf, a persisting current pattern which varies chiefly in the extent to which the coastwise movement predominates over the extensive circulatory movement in the central part of the shelf. Our determinations of the surface flow and volume transport show that

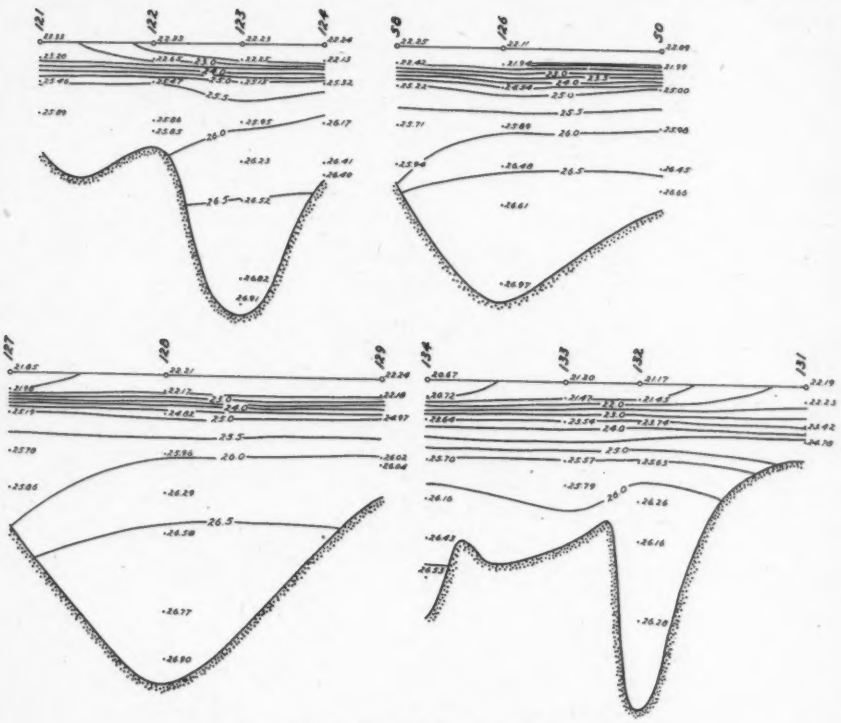

FIGURE 5. Distribution of σ_t in section, August, 1938.

these coastwise movements are chiefly southwesterly, while the central circulatory motion may be cyclonic or anticyclonic. In any event, hydrographic conditions on the Scotian shelf, in any season or year, will be dependent in part upon which of these predominent water movements feature the current pattern at that time.

OFFSHORE CONTRIBUTIONS TO THE SCOTIAN SHELF

It is possible, by means of the network of stations of figure 1, to divide the Scotian shelf into three areas as follows: (*a*) an eastern area, bounded east and west by sections 134-131 and 127-129 respectively; (*b*) a central area bounded east and west by sections 127-129 and 58-50 respectively; and (*c*) a western area bounded east and west by sections 58-50 and 121-124 respectively.

Upon assumption that vertical transference of water particles is negligible in comparison with horizontal transference, and by giving due consideration to conventional signs to indicate direction of flow, the water transport to or from

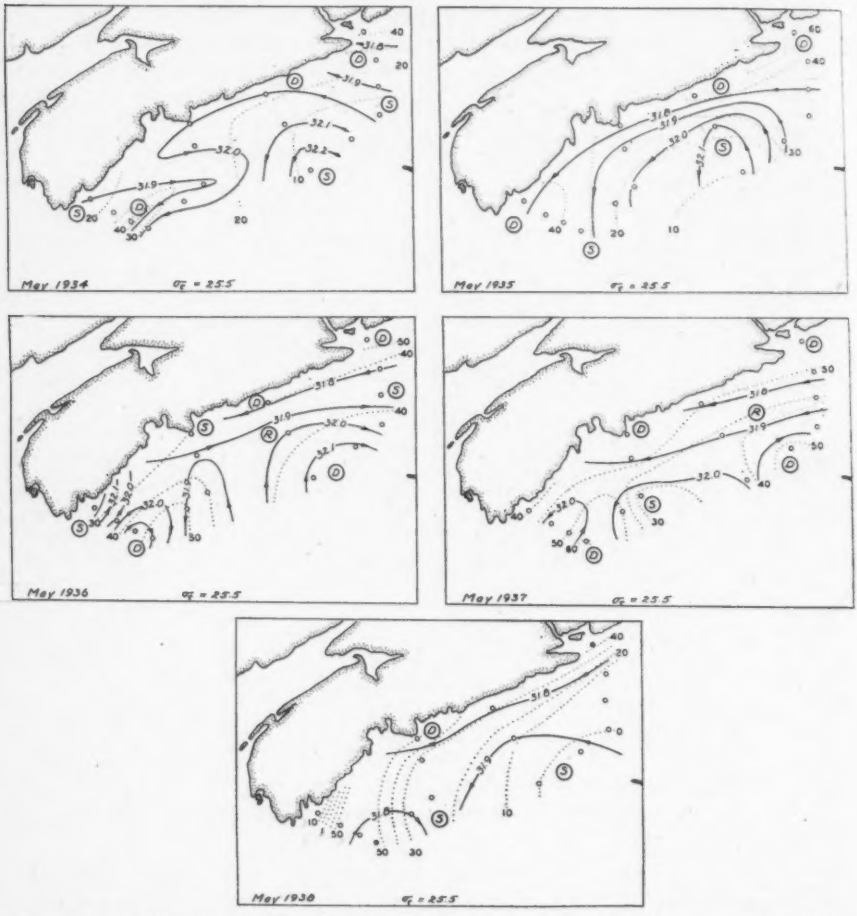

FIGURE 6. Isohalines in a σ_t-surface of 25.50. Depths of σ_t-surface in metres indicated by isobaths (dotted lines).

offshore, in the upper thirty metres, may be readily calculated for each of the three areas as follows: (*a*) for the western area, by a subtraction of the volume transport through the upper thirty metres of section 121-124 from the corresponding volume transport of section 58-50; (*b*) for the central area by a subtraction of the volume transport through the upper thirty metres of section 58-50 from the corresponding volume transport of section 127-129; and (*c*) for the eastern area

by a subtraction of the volume transport through the upper thirty metres of section 127-129 from the corresponding volume transport of section 134-131.

These volume transports, as thus calculated, are listed in table III as gains or losses from offshore through sections 124-50, 50-129, and 129-131 respectively.

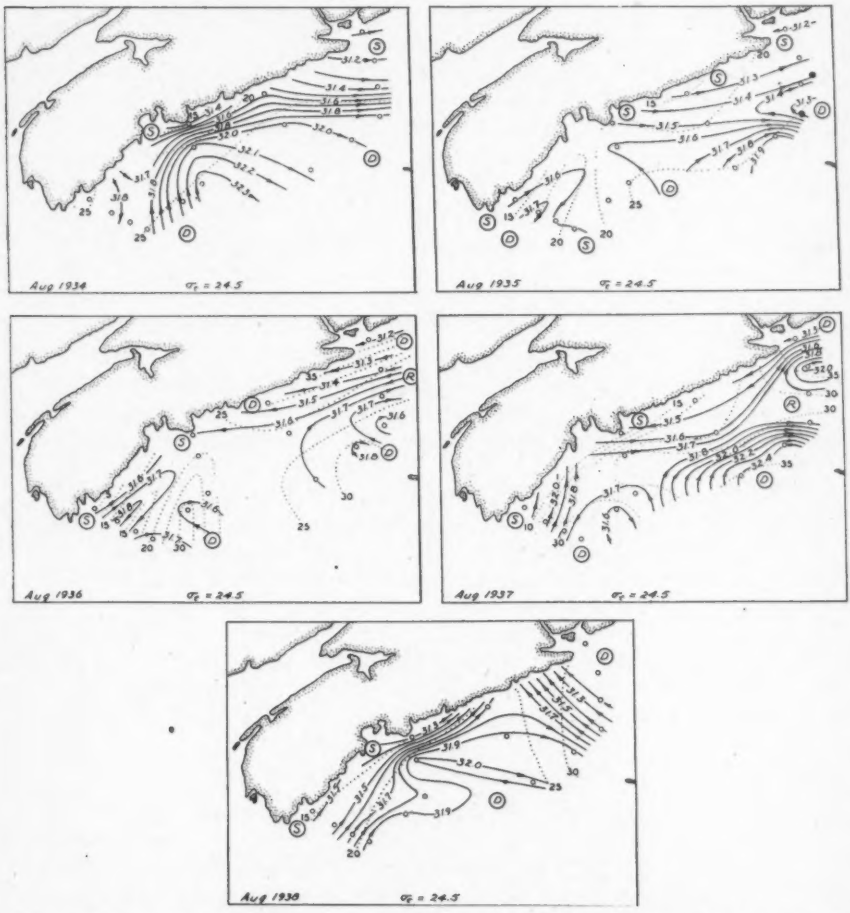

FIGURE 7. Isohalines in a σ_t-surface of 24.50. Depths of σ_t-surface in metres indicated by isobaths (dotted lines).

The total gain (or loss) from offshore is particularly interesting in that in May of 1934, offshore contributions to the area are at the rate of 94,100 cu.m./sec., a figure which is much in excess of the May contributions of other years of the period concerned. With the exception of 1938, the August total was a loss from the Scotian shelf, the rate of transfer being particularly large in 1936 and 1937

(over 100,000 cu.m./sec.). In February, 1937, a large total volume transport to the Scotian shelf was experienced from offshore, while in January, 1938, the transport was only one third as large and in the opposite direction.

TABLE III. Gain or loss to (or from) area.

	Gain (+) or loss (−) to (or from) area in m³/sec.			
Date	124-50	50-129	129-131	Total
1934				
	+	−	+	+
May	78,300	53,100	68,900	94,100
	−	+	−	−
Aug.	37,700	140,100	169,200	66,800
1935				
	−	+	+	−
May	143,700	19,500	114,900	9,300
	−	+	−	−
Aug.	17,800	13,900	31,800	35,700
1936				
	−	−	−	−
May	26,700	27,700	1,500	55,900
	−	+	−	−
Aug.	28,800	35,400	115,000	108,400
1937				
	−	+	+	+
Feb.	49,600	206,300	9,100	165,800
	−	+	+	+
May	107,600	78,500	37,500	8,400
	+	−	−	−
Aug.	40,400	107,100	49,140	115,840
1938				
	−	−	+	−
Jan.	154,600	7,000	106,900	54,700
	−	+	+	+
Ap.-May	154,000	7,800	153,400	7,200
	−	+	+	+
Au.-Sep.	41,300	25,000	69,700	53,400

The figures for the gain or loss to (or from) the central area (section 50-129) are also of peculiar interest in that the August rate of 140,000 cu.m./sec. in 1934, is much greater than corresponding volume transports of other years of the period. The August loss from the central area, at the rate of 107,000 cu.m./sec. in 1937

is of interest also, as these calculated transferences from this central area assist in explaining the special hydrographic features observed in 1934 and 1937 (Leim and Hachey 1935; Hachey 1936, 1937, 1938).

SUMMARY

Detailed water transports, for the upper thirty metres on the Scotian shelf, have been calculated from the hydrographic data of twelve seasonal cruises, collected over a network of stations, within the period 1934-38 inclusive. The volume transport was greatest during the winter season. There may be considerable variation from year to year, and from season to season.

Seasonal surface currents have also been calculated in detail from the hydrographic data. As calculated, these currents ranged from zero to a maximum of 37.9 cm./sec. (18.2 nautical mi./day). The strength of the surface current seemingly has no relation to season, the stronger surface currents being of a temporary nature only.

Methods of isentropic analysis have been used to determine seasonal current patterns. A persisting pattern is indicated, defining a water movement parallel to the coast, a large-scale circulatory movement over the central portion of the shelf, and a complicated vortex in the southwesterly portion of the area. Hydrographic conditions on the Scotian shelf, in any season or year, will be dependent in part upon which of these predominant water movements feature the current pattern at that time.

The seasonal water transports on the Scotian shelf, to and from offshore in the upper thirty metres, have been calculated. The results indicate that, in May, 1934, offshore contributions to the area were at the rate of 94,100 cu.m./sec., a figure which is much in excess of the May transport from offshore during other years of the period 1934-38. In August of the same year, the volume transport from offshore to the central area of the shelf was at the rate of 140,100 cu.m./sec., a much greater August rate than experienced in other years of the period considered. In August, 1937, the volume transport from the central area in the upper thirty metres was at the rate of 107,000 cu.m./sec.

DISCUSSION

The methods of current calculations used herein are based on Bjerknes' circulation theorem. In applying the circulation theorem to ocean waters, to obtain absolute current strengths and volume transports, the following assumptions are made: (*a*) a steady state exists, and therefore no acceleration of currents, and (*b*) there is a depth of no motion. Such conditions are approximated to in the open ocean, but over areas of the continental shelf, the assumption of such an approximation may not be justifiable.

The methods of isentropic analysis, used herein to determine current patterns, are based too upon the approximation of the steady state. Hence, the significance of such current patterns is dependent upon the closeness of the approximation.

It is considered that the use of the above methods on the Scotian shelf is justified, in that at times the steady state can be approximated. Although it is

questionable that a depth of no motion is ever attained, this factor affects only the calculations, in that values for current strengths and volume transports are relative rather than absolute. This justification is based upon the following facts:

(*a*) the waters of the Scotian shelf are highly stratified and very stable throughout the late spring and summer (Hachey 1935, and 1942);

(*b*) the tidal amplitude from cape Sable to Canso and Sable is. varies between 3.8 ft. (1.1 m.) at neaps to 6.5 ft. (2.0 m.) at springs, and the resultant tidal flow is correspondingly small (Tide Tables for the Atlantic Coast of Canada);

(*c*) internal waves are not an important feature of the area, except at times of major disturbances (Hachey 1935);

(*d*) the area of investigation was generally covered in a period of comparatively calm, steady weather and within six days. Upon two occasions, ten days were involved, when four days intervened between the completion of the western half and the beginning of the eastern half of the network. Adjoining stations in the network were occupied within five hours of one another. Calculations are based on adjoining pairs of stations;

(*e*) the calculated winter volume transports are consistently high for sections 58-50 and 127-129, and to the S.W. Such large volume transports are required for the production of the "cold water layer of the Scotian shelf" (Hachey 1938);

(*f*) the correlations, indicated in previous paragraphs, between the contributions to (and from) the Scotian shelf in 1934 and 1937, and the biological and physical phenomena associated therewith: and

(*g*) the persistent current pattern obtained by isentropic analysis, which pattern may be correlated with that obtained from the horizontal distribution of temperatures and salinities (Hachey 1942).

On the basis of the foregoing it is considered that the presentation of the detailed results of the calculations in the various tables is justified, and although not absolutely correct in all details, they allow for certain broad conclusions in keeping with the general hydrographic picture already presented (Hachey 1942).

REFERENCES

HACHEY, H. B. *J. Biol. Bd. Can.*, **1** (4), 227-237, 1935.
Science, **83**, 2154, 1936.
Trans. Amer. Fish. Soc., **66**, 237-241, 1937.
Trans. Roy. Soc. Can., 3rd Ser.. **32**, 29-42, 1938.
J. Fish. Res. Bd. Can., **5** (4), 377-397, 1942.

KNUDSEN, M. Hydrographical tables, Copenhagen, 1-83, 1901.

LEIM, A. H., AND H. B. HACHEY. *Trans. Amer. Fish. Soc.*, **65**, 279-283, 1935.

MONTGOMERY, R. B. *Pap. Phys. Oceanogr. Meteor.* **6**, 2, 1938.

Tide Tables for the Atlantic Coast of Canada. *Can. Dep. Mines and Res.* 1-109, 1944.

Action of Enterobacteriaceae on Choline and Related Compounds

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(*Received for publication April 8, 1946*)

ABSTRACT

The ability of certain of the Enterobacteriaceae to produce trimethylamine from choline has been demonstrated. This provides an additional biochemical test for bacterial taxonomy.

In recent years choline has become recognized as an essential dietary component and its role in the nutrition and metabolism of various animal species has been intensively studied (Best and Huntsman 1932, Kaplan and Chaikoff 1937, Griffith and Wade 1939, Chandler and du Vigneaud 1940). Frequent reference is made to the bacterial degradation of this compound (Waksman 1932, Buchanan and Fulmer 1930, Plimmer 1933, Gortner 1938) despite the fact that few carefully controlled investigations with pure cultures in well defined media have been carried out. Gulewitsch (1898) suggested without experimental evidence that microorganisms would decompose choline and neurine. Supplee (1919), Cusick (1920), Sommer and Smit (1923) found that *Bacillus ichthyosmius* produced trimethylamine from the choline in lecithin. Poller (1933) using a mixed culture obtained from decomposing pancreas, reported trimethylamine, ethyl alcohol, and neurine as products of choline decomposition.

The present study is a continuation of other work on the formation of trimethylamine from its oxide by members of the Enterobacteriaceae (Wood and Baird 1943; Wood, Baird and Keeping 1943). A part of the findings to be reported here have already been published as a note (Wood and Keeping 1944).

EXPERIMENTAL

Cultures

Most of the cultures were those which had been used in the trimethylamine oxide investigation (Wood and Baird 1943). In addition 126 strains from the colon-aerogenes group were freshly isolated from Halifax harbour water and from cod fillets. The cultures were checked for purity from time to time during the course of the investigation.

Medium

The following medium has been found suitable for most species of the Enterobacteriaceae, although certain of the *Erwinia* did not grow well in it: Bactopeptone 5 g., $MgSO_4.7H_2O$ 1g., NaCl 5 g., K_2HPO_4 1 g., choline HCl 5 g., and

J. Fish. Res. Bd. Can., 7 (1) 1947.
Printed in Canada.

distilled water 1000 c.c. Choline hydrochloride (Eastman Kodak Co.) was used. The other inorganic constituents were c. p. quality. The pH of the medium was 7.4 before autoclaving. After autoclaving at 11 pounds pressure for 25 minutes the pH was 7.2.

Choline Decomposition

It was found that trimethylamine was produced by the bacterial decomposition of choline. *Shigella alkalescens* grown in the above medium with excess $CaCO_3$ at 37°C. for 20 hours produced 1950 mg. trimethylamine per litre or 90 per cent of the theoretical yield of 2107 mg. Organic acid was also produced from the choline, unbuffered choline broth changing from pH 7.2 to pH 4.5 in seven hours at 37°C. Trimethylamine production was markedly inhibited by the presence of an available carbohydrate in the medium. Glucose, 0.1 per cent, inhibited the trimethylamine production 50 per cent, and with 0.2 per cent glucose only 25 per cent of the trimethylamine formed in the base medium was obtained. These findings suggest that in the bacterial metabolism of choline the alcoholic side chain is oxidized as a source of energy and the trimethylamine set free as a by-product of the splitting of the molecule. This is in contrast to the role of choline as donor of methyl groups in animal metabolism (du Vigneaud, *et al.* 1941).

Trimethylamine was determined colorimetrically (Dyer 1945) and by vacuum distillation (Beatty and Gibbons 1937). Trimethylamine was also isolated as the hydrochloride from *Shigella alkalescens* cultures in the basal medium. Routine determinations were made by the qualitative trimethylamine method of Wood and Baird (1943). Cultures were always grown in the base medium devoid of choline as negative controls, and choline broth cultures of *Shigella alkalescens* were used as positive controls. At no time was a positive test obtained in the absence of choline in the broth.

Growth Conditions

The various species were grown at their optimum temperature until adequate growth was obtained as judged by turbidity. It was usually found that trimethylamine could readily be detected by the qualitative test after 24 hours incubation. Certain species such as *Shigella alkalescens* gave positive tests after only 12 hours growth.

RESULTS

From one to six strains of the following *Salmonella* were tested: *S. choleraesuis*, *S. schottmuelleri*, *S. typhimurium*, *S. abortivoequina*, *S. abortus ovis*, *S. enteritidis*, *S. paratyphi*, *S. paratyphosa* C, *S. pullorum*, *S. hirschfeldii*, *S. paratyphi* A., *S. anatis*, *S. typhisuis* (Poppe), *S.* sp. (Kentucky), *S.* sp. (Newport), *S.* sp. (London), *S.* sp. (Poona), *S.* sp. (Minnesota) *S.* sp. (Bredeney), *S.* sp. (*Vityphosa*), *S.* sp. (Ballerup), *S.* sp. (Hvittingfoss), *S.* sp. (Onderstepoort), *S.* sp. (Cerre), *S.* sp. (Rubislaw), *S.* sp. (Tel-avi), *S.* sp. (Kerkee), *S.* sp. (Carrau), *S.* sp. (Worthington), *S.* sp. (Urbana), *S.* sp. (Senftenberg), *S.* sp. (Derby), *S.* sp. (Reading), *S.* sp. (Thompson), *S. aertryche*, *S. paratyphi* B. All were negative.

The results obtained with other members of the family Enterobacteriaceae are shown in the table below. "Positive" denotes trimethylamine production from choline. The generic and specific names used in the table are those which the various cultures carried when received at this laboratory.

Species tested	Cultures	
	No. positive	No. negative
Escherichia coli	10	135
" *freundii*	1	12
Aerobacter aerogenes	40	3
Klebsiella ozaenae	0	1
Erwinia amylovora	1	21
Serratia marcescens	0	20
" *indica*	0	2
" *plymouthensis*	1	0
Proteus rettgeri	51	0
" *morganii*	0	9
" *ichthyosmius*	5	0
" *vulgaris*	13	0
" *mirabilis*	12	0
" *hydrophilus*	0	5
Eberthella typhosa	0	13
Shigella alkalescens	84	0
" *dysenteriae*	0	12
" *paradysenteriae*	0	87
" *paradysenteriae* (Boyd)	0	2
" *ambigua*	0	9
" *schmitzii*	0	1
" sp. (Newcastle)	0	5
" *equirulis*	0	1
" *sonnei*	0	27
" *madampensis* (dispar)	0	21
" *ceylonensis*	0	2

DISCUSSION

The genera *Serratia*, *Salmonella*, *Eberthella* and *Erwinia*, with two exceptions, failed to produce trimethylamine from choline. This is in contrast to their ability to produce this amine from trimethylamine oxide (Wood and Baird 1943).

Ninety-three per cent of the *Aerobacter aerogenes* cultures decomposed choline while only six per cent of the *Escherichia coli* strains did so. The division of these two species by means of the choline test appears to be quite sharp. Further work with a greater number of strains of each species might reveal the proper position of the exceptions in this group. Stuart, *et al.* (1943) obtained an almost perfect biochemically intergrading series of cultures from *Shigella alkalescens* to *Escherichia coli* illustrating what appeared to be an evolutionary trend of *S. alkalescens*. The production of trimethylamine from choline by all *S. alkalescens* strains and some strains of the coliform group is further evidence of a close relationship between *S. alkalescens* and this group.

If the reclassification of *Proteus hydrophilus* as *Pseudomonas punctata* (Guthrie and Hitchner 1943) or as *Aeromona hydrophila* (Stanier 1943) or the removal of *P. hydrophilus* and *P. ichthyosmius* from the genus *Proteus* (Borman, Stuart and Wheeler 1944) is granted, then *Proteus morganii* is the only member of the genus *Proteus* which produces trimethylamine from choline.

In the genus *Erwinia* a single positive culture was obtained out of 22 tested. The members of this genus grew poorly in the test medium and for this reason it is suggested that further work should be undertaken before definite conclusions are drawn.

Betaine and acetyl choline bromide were tested with choline positive cultures. In no case were any of the species tested able to produce trimethylamine from betaine. The same results were obtained with acetyl choline bromide as with choline. The *Proteus* group grew well in the acetyl choline bromide broth but a number of strains of several *Shigella* species grew scantily or not at all. Further work on the compounds related to choline is necessary.

Attempts were made to "train" negative cultures of *Proteus morganii*, *Shigella ambigua*, *S. dysenteriae*, *S. paradysenteriae*, *S. sonnei* and *S. gallinarum* by 60 serial transfers in choline broth. In no case did a negative culture become positive under this treatment. Positive cultures carried in broth devoid of choline did not lose their ability to decompose this compound.

The presence of bacteria of this family on fish and fish products is frequently noted. Since certain of these are able to produce trimethylamine from choline it is possible that part of the trimethylamine arising in fish spoilage may come from this source although the carbohydrate present in fish muscle would probably suppress the reaction. Choline occurs in cod muscle to the extent of 78 mg. per 100 g. (Fletcher, Best and Solandt 1935). Choline is thus a potential source of approximately 9 mg. trimethylamine nitrogen per 100 g. cod muscle. Since fourteen times as much trimethylamine nitrogen may be produced from the trimethylamine oxide present (Beatty 1939) and since a larger number of the contaminants usually present on fish reduce trimethylamine oxide (Wood and Baird 1943; Baird and Wood 1944) than decompose choline, trimethylamine production from fish muscle choline is probably of little practical significance.

The demonstration that certain of the intestinal bacteria are capable of decomposing choline would suggest that close attention be paid to the possible interference of bacteria in nutritional experiments with choline.

SUMMARY

Certain members of the family Enterobacteriaceae produced trimethylamine from choline. All strains of *Proteus rettgeri*, *P. ichthyosmius*, *P. vulgaris*, *P. mirabilis* and *Shigella alkalescens* were positive. All strains of *P. morganii*, *P. hydrophilus*, *Salmonella*, *Eberthella*, and all *Shigella* except *S. alkalescens* were negative. Exceptions were encountered in *Escherichia*, *Aerobacter*, *Serratia* and *Erwinia*.

No trimethylamine was obtained from betaine.

The same results were obtained with acetyl choline bromide as with choline.

REFERENCES

BAIRD, E. A., AND A. J. WOOD. *J. Fish. Res. Bd. Can.*, **6**, 243-244, 1944.

BEATTY, S. A., *J. Fish. Res. Bd. Can.*, **4**, 229-232, 1939.

BEATTY, S. A., AND N. E. GIBBONS. *J. Biol. Bd. Can.*, **3**, 77-91, 1937.

BEST, C. H., AND M. E. HUNTSMAN. *J. Physiol.*, **75**, 405-412, 1932.

BORMAN, E. K., C. A. STUART, AND K. M. WHEELER. *J. Bact.*, **48**, 351-367, 1944.

BUCHANAN, R. E., AND E. I. FULMER. Physiology and biochemistry of bacteria. vol. III, 1-575, Williams and Wilkins Co., Baltimore, 1930.

CHANDLER, J. P., AND V. DU VIGNEAUD. *J. Biol. Chem.*, **135**, 223-229, 1940.

CUSICK, J. T. *J. Dairy Sci.*, **3**, 194-205, 1920.

DYER, W. J. *J. Fish. Res. Bd. Can.*, **6**, 351-358, 1945.

FLETCHER, J. P., C. H. BEST, AND O. M. SOLANDT. *Biochem. J.*, **29**, 2278-2284, 1935.

GORTNER, R. A., Outlines of biochemistry, 2nd. ed., 1-1017, John Wiley & Sons Inc., New York, 1938.

GRIFFITH, W. H., AND N. J. WADE. *J. Biol. Chem.*, **131**, 567-577, 1939.

GULEWITSCH, W. *Z. Physiol. Chem.*, **24**, 511-541, 1898.

GUTHRIE, R., AND E. R. HITCHNER. *J. Bact.*, **45**, 52, 1943.

KAPLAN, A., AND I. L. CHAIKOFF. *J. Biol. Chem.*, **120**, 647-657, 1937.

PLIMMER, R. H. A., AND V. G. PLIMMER. Food, health, vitamins. 5th. ed., 1-143, Longmans, Green & Co. Ltd., New York, 1933.

POLLER, K. *Z. Physiol. Chem.*, **217**, 79-82, 1933.

STANIER, R. Y. *J. Bact.*, **46**, 213-214, 1943.

STUART, C. A., R. RUSTIGIAN, A. ZIMMERMAN, AND F. V. CORRIGAN. *J. Immunol.*, **47**, 425-437, 1943.

SOMMER, H. H., AND B. J. SMIT. *Agri. Exper. Sta. Univ. Wis. Res. Bull.* **57**, 1-51, 1923.

SUPPLEE, G. C. *Cornell Univ. Exper. Sta. Mem.* **29**, 101-150, 1919.

DU VIGNEAUD, V., M. COHN, J. P. CHANDLER, J. R. SCHENCK, AND S. SIMMONDS. *J. Biol. Chem.*, **140**, 625-641, 1941.

WAKSMAN, S. A. Principles of soil microbiology, 2nd. ed., 1-894, Williams and Wilkins Co., Baltimore, 1932.

WOOD, A. J., AND E. A. BAIRD. *J. Fish. Res. Bd. Can.*, **6**, 194-201, 1943.

WOOD, A. J., E. A. BAIRD, AND F. E. KEEPING. *J. Bact.*, **46**, 106-107, 1943.

WOOD, A. J., AND F. E. KEEPING. *J. Bact.*, **47**, 309-310, 1944.

Food of Killifish and White Perch in Relation to Supply

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(*Received for publication May 15, 1946*)

ABSTRACT

Killifish (*Fundulus diaphanus*) in Tedford lake, Nova Scotia, selected bottom microcrustaceans, *Hyalella*, chironomids and *Chaoborus*, but not *Amnicola* commensurately with its abundance. They ignored available pelagic microcrustaceans. White perch (*Morone americana*) took pelagic microcrustaceans, *Hyalella*, odonates, chironomids, *Chaoborus*, insect imagines, and small fish, but avoided mollusks. With increase in length, even within narrow limits, they selected larger organisms. About 70 per cent by count and 10 per cent by dry weight of the macroscopic bottom fauna consisted of species eaten by both fish. About 20 per cent by count of the fish population was available to the perch. Other macroscopic bottom organisms and tadpoles were too large or secretive to be available. Available and acceptable bottom organisms were effectively utilized by both fish. Perch cropped the pelagic microcrustaceans poorly, but the available fish effectively.

The treatment of Tedford lake, Nova Scotia, on July 31, 1936, with copper sulphate for the purpose of destroying the undesirable fish population made available a large number of fish for study (Smith 1940). Of the seven species of fish in the lake, specimens of two, killifish (*Fundulus diaphanus diaphanus* (LeSueur)) and white perch (*Morone americana* (Gmelin)), numerically dominant in the population, were selected as representatives of a non-piscivorous and a piscivorous species respectively, and analyses were made of the food contained in their stomachs. Within the week prior to the treatment of the lake with poison, quantitative samples of the pelagic zooplankton and the macroscopic bottom fauna were obtained. It was thus possible to study the dietary preferences of the two species of fish and to assess the extent to which they made use of the food supply as contained in the standing crops of zooplankton, bottom fauna and fish, which seems never to have been attempted.

TEDFORD LAKE

Tedford lake is a small, shallow body of water lying in a region of Precambrian quartzites and slates in Yarmouth county, southwestern Nova Scotia. Certain characteristics of the lake are as follows:

Area	21.05 hectares (52.0 acres)
Volume	435.7×10^3 cu.m. (15.4×10^6 cu.ft.)
Maximum depth	4.7 m. (15.4 ft.)
Mean depth	2.1 m. (6.9 ft.)
Area covered by rooted aquatics (0-2 m.)	42.5 per cent

J. Fish Res. Bd. Can. 7 (1) 1947.
Printed in Canada.

Colour of water (June).....52 (platinum-cobalt standard)
Range in pH values.....5.6 (spring) to 6.6 (summer)
Maximum temperature (Aug. 10, 1938).....22.35°C.(surface); 20.0°C. (bottom)
Dissolved oxygen content (Aug. 10, 1938).....5.55 ml. per 1. (surface); 3.76 ml. per 1. (4.5 m.)

TABLE I. Percentage frequency of microcrustaceans in the pelagic plankton and in the stomachs of killifish and white perch (to 15.0 cm. in length).

	In plankton	In stomachs of fish				
		Killifish	White perch (lengths in cm.)			
		All lengths	7.0–9.0	9.0–11.0	11.0–13.0	13.0–15.0
Pelagic forms			93.7	70.0	65.4	4.5
Diaphanosoma leuchtenbergianum	20.7	—	16.6	31.7	37.0	—
Holopedium gibberum	0.7	—	1.3	4.9	9.5	—
Daphnia longispina hyalina	1.6	—	6.9	2.5	2.6	—
Ceriodaphnia quadrangula	1.6	—	38.2	11.8	4.9	—
Bosmina longirostris	15.1	—	12.1	6.5	2.7	—
Polyphemus pediculus	0.1	—	0.1	—	—	—
Leptodora kindtii	—	—	—	0.1	—	—
Epischura lacustris	0.1	—	1.1	0.3	0.1	—
Diaptomus minutus	51.0	—	10.4	1.7	2.1	—
Mesocyclops obsoletus	9.3	—	7.0	10.5	6.5	4.5
Littoral or bottom forms			6.3	30.1	34.5	95.5
Sida crystallina	—	—	0.1	0.7	1.3	—
Ophryoxus gracilis	—	8.9	0.4	2.7	1.6	6.8
Parophryoxus tabulatus	—	—	—	0.1	—	—
Acantholeberis curvirostris	—	69.5	3.8	25.2	30.4	40.9
Eurycercus lamellatus	—	—	—	—	0.1	—
Acroperus harpae	—	—	0.3	0.3	—	2.3
Alona quadrangularis	—	8.9	0.6	0.5	0.6	—
Chydorus flaviformis	—	1.2	0.1	0.4	0.2	—
C. bicornutus	—	1.8	—	—	—	—
C. sphaericus	—	—	0.8	0.1	—	—
Canthocamptus sp.	—	6.2	—	—	—	45.5
Ostracod spp.	—	3.4	0.2	0.1	0.3	—
Total number of organisms		325	1887	5061	1383	44
Number of fish		79	42	149	87	13
Average number of organisms per fish		4.1	44.9	34.0	15.9	3.4

STANDING CROPS

PELAGIC MICROCRUSTACEANS

The zooplankton was sampled by a 10-litre plankton trap at five stations and at each metre interval of depth. One station was occupied among the rooted aquatic vegetation, the remainder in the open water.

It can be demonstrated, following the analytical procedure employed by Ricker (1938), that the pelagic microcrustaceans were significantly bunched in their horizontal and vertical distribution. The average number of microcrustaceans per 10 litres (12 samples) was 621, with a standard deviation of 143. The percentage frequency of the several species of cladocerans and copepods in the pelagic plankton is shown in table I. Rotifers and copepod nauplii also occurred, but since there was no evidence that these were taken by either the killifish or white perch they are not included.

An estimate of the dry weight of the microcrustaceans in the pelagic plankton of the lake gave 129.5 kg. or 285.5 lb. The calculations are based upon the dry weight of various plankters given by Birge and Juday (1922).

Bottom Fauna

The bottom fauna was sampled at 33 stations with an Ekman dredge which collected the material from 522.5 sq. cm. (81 sq. in.) of the bottom. Two hauls (1045 sq. cm.) constituted a sample for each station. The samples were sorted immediately through a set of three wire screens (4, 13 and 20 meshes to the inch) and the organisms preserved in neutralized formalin.

As with the pelagic microcrustaceans, the bottom organisms were found significantly bunched in their distribution. In the littoral or vegetative zone the average number of bottom animals per 1045 sq. cm. for 12 stations was 46.6 (standard deviation, 22.1), while for the 21 stations in the open water the average was much lower at 6.8 (standard deviation, 3.8). As a result of these findings the two zones are considered separately, and the percentage frequencies and the numbers of the bottom animals in each are shown in table II. The two metre contour of depth agreed very well with the extension offshore of the rooted aquatic vegetation and is taken as representing the boundary between the two zones.

The dry weight of the bottom animals was determined after desiccation in a drying oven for 48 hours at 58 ± 2°C. The weight of the mollusks was determined without shells. Depending upon the species and size, the dry shell made up 60 (sphaeriids) to 96 (*Elliptio* and *Anodonta*) per cent of the total dry weight of the mollusks (average, 84 per cent). The estimated dry weight of the bottom animals in the littoral zone was 138.3 kg. (304.0 lb.), and in the open-water zone, 125.5 kg. (276.7 lb.).

The screens which were used were not of sufficiently fine mesh to retain microscopic bottom organisms such as cladocerans and copepods. This proved to be unfortunate since these microcrustaceans were found to be important food items in the diet of the killifish and white perch.

Vertebrates

An estimate of the fish population was made by counting the poisoned fish as they came into five measured sections of the shoreline, which together constituted 19.3 per cent of the perimeter of the lake. During the 24-hour period

immediately following the treatment of the lake all the fish collected on two of the areas were preserved in formalin. These fish, numbering 5,152, were measured and weighed. The lengths of fish with forked tails were measured from the snout to the distal end of the shortest rays of the caudal fin; of those with rounded or truncated tails to the distal end of the longest rays. The fish were weighed after preservation in formalin for about three weeks.

As far as could be determined almost all the fish came into the shore while dying or later drifted in when dead. An unknown number of fish survived the poisoning, but subsequent observations by netting and angling indicated that

TABLE II. Percentage frequency of macroscopic bottom organisms in dredge samples and in the stomachs of killifish and white perch (Numbers of organisms in brackets).

	In dredge samples		In stomachs of the fish					
			Killifish	White perch (lengths in cm.)				
	0–2 m. depth (12 sta.)	Over 2 m. depth (21 sta.)	All lengths	7.0–9.0	9.0–11.0	11.0–13.0	13.0–15.0	Over 15.0
Oligochaeta	0.5(3)	—	—	—	—	—	—	—
Hirudinea	0.7(4)	0.7(1)	—	—	—	—	—	—
Hyalella	29.9(167)	12.7(18)	21.5(28)	30.2(32)	39.1(189)	20.3(75)	3.7(1)	—
Asellus	14.7(82)	0.7(1)	—	—	—	0.7(3)	—	—
Sialis	—	2.1(3)	—	—	—	—	—	—
Mayfly nymphs	1.3(7)	7.7(11)	—	—	—	0.7(3)	—	—
Dragonfly nymphs	1.6(9)	2.8(4)	—	—	1.7(8)	5.4(20)	37.0(9)	100.0(9)
Damselfly nymphs	0.4(2)	—	—	—	—	—	—	—
Caddisfly larvae	—	3.5(5)	—	—	—	—	—	—
Chironomid larvae and pupae	9.8(55)	20.4(29)	60.8(79)	17.0(18)	50.9(246)	65.4(243)	40.7(11)	—
Chaoborus larvae and pupae	0.5(3)	26.1(37)	5.4(7)	42.5(45)	5.0(24)	2.3(8)	—	—
Insect imagines	—	—	3.1(4)	9.4(10)	2.3(11)	3.8(14)	—	—
Amnicola	31.7(177)	9.9(14)	9.2(12)	—	—	—	—	—
Other gastropods	2.5(14)	6.3(9)	—	—	0.2(1)	—	—	—
Sphaeriids	4.7(26)	0.7(1)	—	—	—	—	—	—
Anodonta and *Elliptio*	0.9(5)	5.6(8)	—	—	—	—	—	—
Miscellaneous	0.9(5)	0.7(1)		0.9(1)	0.8(4)	1.4(5)	18.5(5)	—
Total number of organisms	559	142	130	106	483	371	29	9
Average number per fish			1.6	2.5	3.2	4.3	2.0	1.1

the number was small. During the period of collection the winds were not sufficiently strong to cause fish to drift in appreciable numbers from one area to another.

The species and estimated number of fish in the lake are recorded in table III. The number of each species for every two centimetres of length is given to 15.0 cm., above which all lengths are grouped. The estimated number of fish was 86,217. The estimated wet weight was 40.3 kg. per hectare or 36.0 lb. per acre.

Frog tadpoles, presumably *Rana catesbiana*, were also killed by the poisoning. Estimates of the number and weight of these were made in the same manner as for the fish. The estimated population in the lake was 10,932. The estimated wet weight was 4.5 kg. per hectare or 4.0 lb. per acre.

TABLE III. The estimated total fish population of Tedford lake.

Length (cm.)	<3.0 -3.0	3.0 -5.0	5.0 -7.0	7.0 -9.0	9.0 -11.0	11.0 -13.0	13.0 -15.0	Over 15.0	Totals
Notemigonus crysoleucas	—	32	1996	2329	3161	372	32	—	7922
Ameiurus nebulosus	—	—	—	44	15	73	176	1383	1691
Anguilla bostoniensis	—	—	—	—	189	299	463	1871	2822
Fundulus diaphanus	85	2728	19649	19094	1065	—	—	—	42621
Perca flavescens	15	2798	295	2681	1159	295	118	22	7383
Morone americana	—	—	—	2633	16561	4010	166	356	23726
Pungitius pungitius	42	10	—	—	—	—	—	—	52
Totals	142	5568	21940	26781	22150	5049	955	3632	86217
Percentages	0.2	6.4	25.4	31.1	25.7	5.9	1.1	4.2	

SUMMARY OF STANDING CROPS

The standing crops of animals, as estimated from the sample data, may be summarized as follows:

Pelagic microcrustaceans
- Number.....621 per 10 l.
- Total dry weight.....129.5 kg.

Macroscopic bottom fauna
- Number.....46.6 and 6.8 per 1045 sq. cm. in the vegetative and open water zones respectively.
- Total dry weight (exclusive of mollusk shells).....138.3 and 125.5 kg. in the same zones respectively.

Fish
- Total number.....86,217
- Total wet weight.....848.3 kg.

Frog tadpoles
- Total number.....10,932
- Total wet weight.....94.7 kg.

USE OF CROPS

METHOD

The stomach contents of the white perch were separated into samples corresponding to the size classes in table III. No food materials beyond the pylorus were included.

With the killifish, which has no true stomach ("siphonal stomach"), the food organisms in the first two loops of the intestine were removed, and all organisms were brought together into one sample.

The organisms in all samples were enumerated as far as was possible.

By this procedure the individuality of the fish in relation to feeding is lost. The presence of a particular food organism in the composite samples of the food contents could result from its occurrence in only one or two fish. For the abundant food organisms, however, an examination of the food in a number of individual specimens demonstrated that the composite samples were quite representative of the food taken by the majority of the fish involved in each sample.

KILLIFISH

The sample of fish for food analysis consisted of 201 individuals of which only 79 specimens (39 per cent) contained food in the first two loops of the intestine. The fish ranged in length from 4.1 to 9.8 cm.

The percentage frequency by count of the food items was as follows: Cladocera, 64.6; Copepoda, 4.4; Ostracoda, 2.4; *Hyalella*, 6.2; insect larvae, 16.7; insect pupae, 2.2; insect imagines, 0.9; *Amnicola*, 2.6.

More detailed analyses of the microcrustaceans and the macroscopic bottom faunal constituents of the food consumed by this species are shown in tables I and II.

Forbes (1883) and Pearse (1918) found that animals of the bottom fauna predominated in the food of *Fundulus diaphanus menona* from Illinois and Wisconsin waters. In Tedford lake the closely allied subspecies, *diaphanus*, was also a bottom feeder. Thus, of the microcrustaceans only the bottom-haunting or littoral species, predominantly *Acantholeberis*, were eaten. Chironomid and *Chaoborus* larvae and pupae, *Hyalella* and *Amnicola* of the macroscopic bottom fauna formed a bulk of the food. The insect imagines consisted of corixids which could be taken by bottom feeding.

Forbes (1883), Hankinson (1908) and Moore (1922) found algae, seeds and other vegetative matter taken by killifish in sufficient quantity for them to conclude that plant material was a consistent part of this species' diet. This conclusion was not borne out in Tedford lake since filamentous algae were a very minor item in the food of seven fish only, and the quantities suggested that the algae were taken incidentally along with other foods.

WHITE PERCH

In all, 773 stomachs from white perch were examined, and 299 (39 per cent) were found to contain food. White perch of the year were not obtained and were apparently not present in the lake. The smallest specimen in the sample had a length of 7.1 cm.

The percentage frequency by count of the food items in the stomachs of the entire sample of white perch was as follows: Cladocera, 77.0; Copepoda, 12.0; Ostracoda, 0.1; *Hyalella*, 3.2; insect larvae, 5.9; insect pupae, 1.0; insect imagines, 0.4; mollusks, <0.1; fish, 0.2; miscellaneous, 0.2.

Further analyses of the food organisms are presented in tables I and II.

Both pelagic and bottom-haunting microcrustaceans were eaten, the former to the greatest extent by the smaller fish, the latter in increasing numbers as the size of the fish increased to 15.0 cm. Above this length the white perch did not take microcrustaceans of any sort. Of the immature insects, odonates, chironomids and *Chaoborus* were encountered most frequently. Corixids were found in the stomachs of fish from the length group of 13.0 to 15.0 cm., and a fair number of dipterous imagines were captured, presumably by surface feeding. Mollusks were almost entirely absent from the white perch stomachs.

Only white perch over 11.0 cm. in length had eaten other fish. In total, 17 fish (13 killifish, 3 yellow perch, 1 stickleback), all under 5 cm. in length, were found

in the stomachs of 13 white perch. Thus there were approximately 5,700 fish in the lake of the size that the white perch were eating (table III). The dry weight of these fish was estimated at 1.2 kg. (2.6 lb.). The calculations are based upon samples of these small fish which were cut up and dried to a constant weight at 60 ± 2°C.

Four white perch (11.0 to 13.0 cm.) had waterlily seeds (*Nuphar*) in their stomachs. Such seeds were also noted in the intestines of these and other specimens in an undigested condition. Presumably the seeds had no food value to the fish. Filaments of algae were found sparingly, especially when the food had been largely taken from the bottom, and it would appear that the white perch had inadvertently ingested the algae along with other items of food.

TABLE IV. Percentages of animals in macroscopic bottom fauna subject to predation by killifish and white perch.

	Killifish	White perch
By count		
Littoral zone	72	61
Open-water zone	69	77
By dry weight		
Littoral zone	10	13
Open-water zone	3	7
By dry weight, *Anodonta* and *Elliptio* excluded.		
Littoral zone	46	60
Open-water zone	27	64

EXTENT OF USE

The extent to which the standing crops were utilized by the killifish and white perch may be discussed under two headings: (1) with regard to all the organisms in the standing crops, and (2) with regard to only those organisms preyed upon by the fish.

(1) The killifish and white perch did not utilize certain animals as food either because they were not available or because the fish were not inclined to eat them. Thus the killifish did not utilize the food supply contained in the pelagic cladocerans and copepods (estimated at 129.5 kg. dry weight), and both this species and the white perch did not take a number of bottom animals which, in aggregate, constituted a considerable portion of the macroscopic bottom fauna. The frog tadpoles and most of the fish were too large for the white perch to consume.

In table IV it is shown that approximately 70 per cent by count, but only about 10 per cent by dry weight of the estimated standing crop in the bottom fauna were of those species or groups of species which entered into the diets of the killifish and white perch. The large mollusks, *Anodonta* and *Elliptio*, which were not eaten by the fish, although not very numerous, were comparatively heavy, and accounted for a considerable part of the weight not utilized. If these animals are excluded from the calculations, the percentages by dry weight of the animals

utilized are materially increased, but the percentage by weight of the bottom fauna taken by the killifish and white perch still remains low.

The data demonstrate that quantitative sampling of the plankton, bottom fauna, and fish life of a lake in an attempt to assess the supply of fish foods loses considerable value unless cognizance is taken at the same time of the actual forms eaten by the fish. Obviously some animals can be judged correctly as too large to be consumed. However, stomach analyses were necessary to bring out, for instance, such facts as that in Tedford lake the killifish did not feed upon the pelagic microcrustaceans and that the white perch avoided mollusks.

(2) Further consideration was given to the quantity of the food eaten by the killifish and white perch. The dry weight of the animals in the stomachs of

TABLE V. Estimated dry weight of animals in stomachs of killifish and white perch populations and of supply of same animals in the lake.

	Supply in lake (g.)	In stomachs of fish (g.)		Ratio In supply: In stomachs of fish
		Killifish	White perch	
Pelagic microcrustaceans.....	129,500	—	1.2	107916 : 1
Macroscopic bottom fauna....	30,458	18.6	23.8	718 : 1
Fish (5 cm. and under)....	1,193	—	142.3	8 : 1

these fish in the sample was determined. Assuming the same proportion of fish in the entire population as in the sample had food in their stomachs and in similar amounts, an estimate was made of the total dry weight of the animals in the stomachs of all the killifish and white perch in the lake. By comparing the dry weight of the animals in the stomachs with that of the same forms in the lake information is obtained upon the extent to which the killifish and white perch cropped the groups of animals upon which they fed. The estimates may be compared in table V. The ratios of the quantities in the supply to those in the stomachs of the fish indicate that the white perch were making no serious inroad into the available supply of pelagic microcrustaceans, but that a different situation obtained with respect to the bottom fauna and small fish. The data signify that the supply of these latter foods, especially that of the small fish, was being effectively cropped. The relative scarcity of fish of 5 cm. in length and under (6.8 per cent) in the total population corroborates this view.

Neither the killifish nor white perch appeared to be feeding vigorously just prior to their death. Regurgitation of food might be offered as an alternative explanation for the absence or scarcity of food in so many stomachs. There is no specific information upon this point. If regurgitation was a factor affecting the quantity of food in the stomachs, the calculations in table V lose much significance, but the effect would be toward a greater cropping than was illustrated.

FACTORS AFFECTING USE

The extent to which the animals in the standing crops in Tedford lake were utilized by the killifish and white perch would be determined by the availability of the organisms and selection by the fish.

Availability of Organisms

The availability of the organisms in Tedford lake as fish foods would be determined by a number of complex factors among which their (1) abundance, (2) size, (3) concealment, and (4) distribution in relation to that of the fish, may be considered more fully.

(1) An organism may not be used as a fish food commensurate with its frequency in the habitat. *Hyalella*, *Asellus*, chironomid and *Chaoborus* larvae and pupae, and *Amnicola* were the most numerous constituents of the macroscopic bottom fauna in Tedford lake (table II). *Hyalella* and the chironomids were also dominant among the foods eaten by both the killifish and white perch. However, the extent to which *Asellus*, *Chaoborus* and *Amnicola* of the bottom fauna, as well as certain of the microcrustaceans of the pelagic plankton, were utilized illustrates that factors other than abundance alone were determinative.

(2) The *large* size of some members of the bottom fauna and of the fish and amphibian populations would make them unavailable to the killifish and white perch. Obviously the mussels, *Anodonta* and *Elliptio*, of the size that occurred in the bottom samples, could not have been eaten by any of the fish in Tedford lake. Likewise, the more fully developed odonate nymphs, and the larger of the gastropods, *Campeloma* and *Helisoma*, and of the leeches, *Macrobdella*, were not available to most, if not all, of the killifish and to the smaller white perch. The white perch did not eat other fish until they were 11 cm., or more, in length and then took only fish that were under 5 cm. in length. Table III shows that a large portion of the fish population consisted of individuals too big for the white perch to ingest. Thus the majority of the white perch were not piscivorous, even if so inclined, since they were smaller than, or approximated too closely to the size of so many of the other fish upon which they might have otherwise fed (82 per cent of the fish in the lake were in the length groups from 5 to 11 cm.). The size of the amphibians also precluded their use as food by most of the white perch.

The *small* size of organisms also determined their availability. It is considered that killifish and white perch feed by sighting their prey and seizing individual organisms. Accordingly, there was a lower limit in size below which the animals were too small to attract attention, even if active, and thus they were essentially unavailable. Rotifers and copepod nauplii were not eaten by the white perch, although these plankters were numerous in the pelagic plankton. This suggests, with regard to the smallest of the white perch present in the lake at that time, that the lower limit in size of the animals upon which these fish would feed lay between that of the rotifers and copepod nauplii on the one hand and the smaller entomostracans, such as *Bosmina* and *Ceriodaphnia*, on the other.

(3) Concealment may be effected by organisms, particularly those of the bottom fauna, if they remain hidden under bottom materials, or, to a varying

degree, if they remain quiescent, even if exposed, since movement undoubtedly attracts fish that feed in the manner of the killifish and white perch. Concealment as a factor in availability is difficult to assess however, since the intimate ecological relationships of the small bottom forms are poorly understood. Oligochaetes and sphaeriids are usually well hidden and probably for this reason were not taken by the fish. The absence of *Asellus* from the food of the killifish, although this crustacean was comparatively plentiful in the littoral zone, might be attributed to secretiveness and slow movements.

(4) The utilization of an organism as a fish food is contingent upon its occurrence in the same part of the habitat as that of the fish. The typical habitat of the killifish in summer is the littoral zone of lakes, but the white perch, on the other hand, is a species of the more open water. The two species are thus largely segregated in limnetic environments by habitat preferences. However, in a lake as small and shallow as Tedford where water conditions, such as temperature, are quite similar throughout, and present no barrier to the movements of fish, the killifish and white perch may be expected to intermingle considerably. There is evidence for this in the fact that the white perch fed upon the killifish. Nevertheless, in view of the habitat preferences of the two fish, there is justification in considering the food consumed by the killifish more with respect to the supply in the littoral vegetative zone than to that in the open water, and in reverse manner in the case of the white perch, thereby implying that the food supply in either the littoral or open-water areas was not equally available to the two species. As an instance, *Chaoborus* larvae and pupae were most plentiful in the deepest portions of the lake, and, as a result, would presumably be more available to the white perch than to the killifish. Actually this contention is borne out by finding these insects most frequently in the food of the perch.

Selection of Food

In the above sections an attempt was made to show that various organisms in Tedford lake were not available, or poorly so, for a number of reasons, and therefore were absent, or were found sparingly, in the fish stomachs. However, the data demonstrate instances of available food animals not being eaten by the killifish and white perch in proportion to their frequency of occurrence in the lake, indicating selection on the part of these fish. No selection of food is indicated if the percentages of the food animals in the stomachs of the fish are the same as the percentages of these animals in the fauna of the lake, or if there is an approximation to equality since discrepancies between the percentages are expected to result from sampling errors.

Killifish

The disposition of the killifish to feed upon animals at or near the bottom was apparently a strong one as illustrated by the discrimination that they made between the bottom-haunting and the more pelagic microcrustaceans (table I). Sampling with the plankton trap in shallow water (less than one metre) among the aquatic vegetation demonstrated that the cladocerans and copepods of the

pelagic plankton also occurred plentifully in those locations frequented by the killifish. It is assumed therefore that if the killifish had been inclined to feed not only at the bottom but also in the water above, both groups of microcrustaceans would have been available to them.

In table II it is shown that the percentages which the food animals of the bottom fauna made up of the food of the killifish lack agreement for the most part with their percentages in the lake. *Asellus* and some others have been discussed and deemed unavailable to the killifish. Of the bottom organisms which were most numerous and available, *Hyalella* had percentages of occurrence in the fish stomachs and in the habitat approximating each other more closely than was the case with chironomids, *Chaoborus* and *Amnicola*. If primary consideration is given to the bottom fauna in the littoral zone, where the majority of the killifish are assumed to have been, it appears that the killifish definitely selected the chironomids, possibly to some extent *Chaoborus*, but, on the other hand, tended to avoid *Amnicola*.

White Perch

The extent to which the white perch utilized the available food animals varied in relation to the size of the fish, even within rather narrow limits of length. For instance, the percentage compositions of the food eaten by the two length groups containing the smaller fish (7.0 to 9.0 and 9.0 to 11.0 cm.) were quite different although the same food animals were involved. Since it is reasonable to expect that the food organisms were as accessible to the fish in the one group as to those in the other, the conclusion appears warranted that the white perch exercised considerable selection in their feeding. It was observed, however, that, in general, the larger the fish the more prone were they to eat larger organisms.

The few white perch in the sample that were over 15 cm. in length had only other species of fish and large dragonfly nymphs in their stomachs. Within the length range from 7 to 15 cm. the white perch fed extensively upon microcrustaceans, but the average number of these per stomach decreased with increase in the size of the fish (table I). Paralleling this, the average number per stomach of the comparatively large bottom animals increased until the length of the perch was about 13 cm., at which length fish were already included in, and the smaller invertebrates being dropped from the diet (table II). With reference to the microcrustaceans, the white perch became more bottom feeders with increase in size, as indicated by the greater proportion of bottom forms, especially *Acantholeberis*, in their food. The smallest white perch fed liberally upon the smallest microcrustaceans, such as *Ceriodaphnia*, *Bosmina* and *Diaptomus*, while fish of a few more centimetres in length consumed less of these and more of the somewhat larger *Diaphanosoma* and *Acantholeberis*, although there was only a difference in size of about 2 mm. in length between the smallest and the largest of these microcrustaceans.

Notably absent from the food in the stomachs of the white perch were *Amnicola* and other available mollusks. The white perch appeared to ignore them almost entirely. Similarly, Cooper (1941) found that white perch in a number of

Maine lakes apparently avoided mollusks although these were relatively abundant in the bottom fauna of the lakes and were of sufficiently small size to be ingested.

SUMMARY

Estimates were made of the mid-summer standing crops of pelagic microcrustaceans, microscopic bottom fauna, frog tadpoles and fish in Tedford lake, Nova Scotia, and a study made of the utilization of these as food by killifish and white perch.

The killifish were bottom feeders and took bottom and littoral microcrustaceans, and *Hyalella*, chironomids, *Chaoborus* and *Amnicola* of the macroscopic bottom fauna. The white perch took pelagic and bottom-haunting microcrustaceans, *Hyalella*, odonates, chironomids, *Chaoborus*, insect imagines and small fish.

Approximately 70 per cent by count and 10 per cent by dry weight of the estimated standing crop of the bottom fauna consisted of those species or groups of species which were eaten by the killifish and white perch. About 20 per cent by count of the fish population were individuals sufficiently small for the white perch to ingest. Mussels, the larger snails and leeches, *Asellus*, oligochaetes, sphaeriids and frog tadpoles were apparently too large or secretive to be available as food.

Both the killifish and white perch exercised selection in feeding upon the available organisms. The killifish did not take the available pelagic microcrustaceans, nor *Amnicola* commensurately with its occurrence, but selected chironomids and *Chaoborus*. The diet of the white perch changed with increase in size, even within rather narrow limits of length, with a tendency toward a selection of larger organisms. The available mollusks were almost entirely ignored by the white perch.

A comparison of the dry weight of the organisms in the stomachs of killifish and white perch with that of the same kinds of organisms in the lake indicated that the pelagic microcrustaceans were poorly cropped by the white perch, while the available and acceptable macroscopic bottom organisms were effectively utilized by both fish, as was the available fish population by the white perch.

It was demonstrated that concurrent studies of the standing crops and of the food in the stomachs of the fish were necessary to evaluate properly the supply of fish foods in the plankton, bottom fauna and fish population of a lake.

REFERENCES

BIRGE, E. A., AND C. JUDAY. The inland lakes of Wisconsin. The plankton, I. Its quantity and chemical composition. *Bull. Wis. Geol. Nat. Hist. Surv.*, **64**, I-IX, 1-222, 1922.

COOPER, G. P. A biological survey of lakes and ponds of the Androscoggin and Kennebec river drainage systems in Maine. *Me. Dept. Inland Fish. Game., Fish. Surv. Rep.*, **4**, 1-238, 1941.

FORBES, S. A. The food of the smaller freshwater fishes. *Bull. Ill. State Lab. Nat. Hist.*, **1** (6), 61-86, 1883.

HANKINSON, T. L. A biological survey of Walnut lake, Michigan. *Rep. Mich. State Bd. Geol. Surv.*, **1907**, 157-288, 1908.

MOORE, J. P. Use of fishes for control of mosquitoes in northern fresh waters of the United States. *Rep. U. S. Comm. Fish.*, **1922**, App. IV., 1-60, 1922.

PEARSE, A. S. The food of the shore fishes of eastern Wisconsin lakes. *Bull. U. S. Bur. Fish.*, **35**, 245-292, 1918.

RICKER, W. E. On adequate sampling of the pelagic net plankton of a lake. *J. Fish. Res. Bd. Can.*, **4** (1), 19-32, 1938.

SMITH, M. W. Copper sulphate and rotenone as fish poisons. *Trans. Amer. Fish. Soc.*, **69**, 141-157, 1940.

The Nutritive Value of Marine Products
XVI. The Biological Value of Fish Flesh Proteins

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(*Received for publication June 20, 1946*)

ABSTRACT

The biological values of the crude flesh proteins of lingcod, halibut, lemon sole, and white spring salmon have been determined by growth and nitrogen retention methods using young Wistar rats as experimental animals. Additional values obtained by the growth method are reported for herring and red snapper. For comparative purposes, values were also secured on crude beef flesh proteins, egg albumin, and casein. The figures obtained for the flesh proteins from the four types of fish were of the same order of magnitude, and they were higher than those procured for the other protein foods tested. An examination of the gains in weight was carried out by the co-variance method in which the variance of the actual gains, freed from the effects of varying food consumption by regression, was subjected to analysis. This test indicated that the differences in the average weight gains between the rats fed fish flesh and those fed the other protein foods were highly significant. Data on the effect of sex on the determination of biological values are submitted along with an explanation which helps to resolve conflicting reports on this point.

Recent work demonstrating the deleterious effects of protein deficiency on the healing of wounds (Thompson, Ravdin and Rhoads 1938; Morris, Dubnik and Dunn 1945), resistance to infections (Cannon et al. 1943, 1944), and prevention of chloroform liver injury (Miller, Ross and Whipple 1940) has aroused increased interest in the dietary significance of proteins. Several reviews published in the last year or two deal adequately with these and other aspects of protein nutrition in health and disease (cf. Elman 1944; Stare, Hegsted and McKibbin 1945; and Co Tui 1946). These reports emphasize the desirability of having data readily available on the nutritive value of the main protein food stuffs. A relatively abundant literature exists regarding the proteins of beef, egg, milk, and cereals, but amongst the more important protein foods, least is generally known and published respecting the nutritive properties of fish flesh protiens. Indeed in a number of reference books and reviews on protein nutrition no data whatsoever can be found on these nutrients. In order to supply this information in greater degree the work here reported was conceived and carried out.

At the suggestion of Dr. N. M. Carter, Director of the Pacific Fisheries Experimental Station, four commercially important types of fish were chosen for this study: lingcod (*Ophiodon elongatus*), halibut (*Hippoglossus stenolepis*), lemon

J. Fish. Res. Bd. Can., 7 (1) 1947.
Printed in Canada.

sole (*Parophrys vetulus*), and white spring salmon (*Oncorhynchus tshawytscha*). Additional determinations were subsequently performed on herring (*Clupea pallasii*) and red snapper (*Sebastodes ruberrimus*). Other protein preparations included in the series for comparative purposes were those of beef flesh, egg albumin, and casein. The latter was a vitamin-free product, Labco brand, and the albumin was a pan-dried product from the firm of Canada Egg Products. The beef flesh protein preparation was obtained by processing similar proportions of all the wholesale cuts and was thus roughly representative of the whole steer: round steak 2.5 lb., sirloin 2.5 lb., tenderloin 1.5 lb., stewing meat (flank and shoulder) 6 lb., prime rib roast 2.5 lb. (1 lb. equals 0.45 kg.)

EXPERIMENTAL

The fish were prepared in the form of fillets or steaks and cooked one hour at 99 to 100°C. in a steam autoclave. The cooked product was then pressed, minced, and partially dehydrated in a warm air tunnel at 60°C. The cooking liquors were saved, separated from accompanying oil or fat, and evaporated to a thick syrup in vacuo on a water bath at 40 to 50°C. The concentrate was then re-incorporated into the partially dehydrated minced flesh and the whole re-dried at 50°C. to a moisture content of 2 to 4 per cent. The preparations were then ground into a fine meal. The beef flesh was treated in the same manner except that as much as possible of the fat and discrete connective tissue were discarded before cooking and any observed after cooking was also discarded. This was done since one of the intended features of the investigation was to test only that part of the protein foods which is usually consumed by man. It was assumed that the gross connective tissue did not come under that category. The cooking liquors were retained in the protein preparation because such a procedure simulates best household practice.

In the five feeding tests described below, fresh protein preparations were made for each trial, with the exception of the halibut of trial 5, and the lingcod, lemon sole, and white spring salmon preparations listed in table IV. As a necessary preliminary task, moisture, nitrogen, and fat determinations were carried out on each protein product. The diets (table I) were then made up on the basis of these figures to contain the same amount of crude protein (Nx6.25) and to be of isocaloric value. Nitrogen determinations were carried out on certain of the diets and amounts agreeing closely with the expected values were obtained. The rats were of the Wistar strain and in every case they were divided amongst the different groups according to weight, sex, and litter. They were housed in a room at a controlled temperature of 20 to 22°C. in individual cages having $\frac{1}{4}$-inch mesh screen floors.

The ideal diet utilized to determine the biological value of proteins should be adequate for maximal growth except with respect to protein. Perhaps the most difficult problem confronting anyone attempting to determine the biological value of proteins is the provision of B-vitamins without the introduction of protein material other than that being tested. This obstacle has diminished to some extent in recent years, owing to the preparation of certain of the B-vitamins

in pure form. Evidence is herewith presented to show that under the conditions set forth in this paper, protein is just as efficiently utilized when the following five crystalline vitamins are supplied: thiamine, riboflavin, pyridoxine, calcium pantothenate, and nicotinamide, as when yeast and liver concentrates are supplied as sources of the B-vitamins. In order to demonstrate the adequacy of the basal diet, control diets were fed containing adequate amounts of protein for maximal growth. In three of the feeding trials herein reported, the test protein was fed at a level of 8%, and at 10 and 12% in the remaining trials. The level of 8% was chosen so as to allow a moderate degree of growth which was definitely below the maximum. These conditions have been found to be well suited to the type of work under investigation (Osborne, Mendel and Ferry 1919). A level of 12% test protein was utilized in the fourth feeding trial to determine whether or not the differences in biological values observed at a level of 8% protein were maintained.

Table I shows the composition of the basal (A) and control diets utilized in the feeding experiments.

TABLE I. Percentage composition of diets

Components	A	B	C	D	E	F	G	H
Test protein	8	8	8	12				
Casein (a)					20	20	20	20
Beef dripping	20	20	10	10	20	20	10	10
Corn oil (b)			10	10			10	10
Sucrose	63	62.9	62.3	58.3	51	45	50.3	48.8
Salts (c)	5	5	5	5	5	5	5	5
Agar	2	2	2	2	2	2	2	2
Cod liver oil (d)	1	1	1	1	1	1	1	1
Vitamin mixture (e)	1	1	1	1	1	1	1	1
Yeast						5		
Yeast concentrate (f)			0.25	0.25			0.25	1
Liver concentrate (g)			0.25	0.25		1.0	0.25	1
Choline chloride	*	0.1	0.20	0.20	0.1	0.1	0.20	0.2

(a) Labco brand, vitamin-free. (b) Mazola. (c) (Beveridge and Lucas 1945). (d) Mead's, 1800 I. U. of vitamin A per g. and 175 I.U. of vitamin D per g. (e) The vitamin mixture is made up of powdered sugar and crystalline vitamins (obtained from Merck) so that 10 g. food supplied 25γ thiamine hydrochloride, 40γ riboflavin, 20γ pyridoxine hydrochloride, 100γ calcium pantothenate, 100γ nicotinamide. (f) Harris Laboratories, Tuckahoe, New York. (g) Eli Lilly and Co., Indianapolis, U.S.A. *Choline chloride included in vitamin mixture so that 10 g. food supplied 10 mg. choline chloride.

FEEDING TRIAL 1

The fact that biological values derived from rats with widely different food intakes possess only doubtful comparative significance is one which is well known (Osborne, Mendel and Ferry 1919). An attempt was therefore made to perform

the experiment on a paired feeding basis. Since some preliminary work had indicated that the lemon sole and lingcod diets were perhaps slightly less palatable than the other rations, the animals were paired-fed on their mates eating the lingcod preparation.

TABLE II. Biological values of proteins of fish and beef flesh and of egg albumin. Average initial wt. of rats: 67.6 g. Range: 40-97 g. Days on test diets: 28. No. of rats per group: 10. Basal diet: A. The rats were paired-fed on the corresponding animals eating the lingcod diet which was given ad libitum.

Source of dietary protein	Daily food intake (g.)	Average gain in wt. (g.)	Gain in wt. per g. protein eaten (g.)
Lingcod	9.6	56.3	2.62
Halibut	9.0	50.3	2.47
Lemon sole	9.0	50.1	2.50
Salmon	9.1	52.0	2.56
Beef	9.0	46.6	2.31
Egg albumin	8.7	45.6	2.34
Control diet E	9.0	76.6	—

In the results (table II), an examination of the food intake reveals that the attempted paired feeding technique was not entirely successful. This was due to the fact that the palatabilities of the diets were essentially the same. The rats on the diet that was fed ad libitum, ate from 5 to 10% more food than did those on the other rations and for that reason greater gains were made and higher biological values observed in this group (cf. Osborne et al. 1919). The gains and biological values induced by the three other fish flesh diets were almost identical and greater than those brought about by the beef flesh and egg albumin diets. It may be noted that the rats on control diet E, identical to the test diets except that it contained an adequate amount of protein for growth in the form of 20% vitamin-free casein, made an average gain of 76.6 grams. This indicated that the chief limiting factor in the test diet was protein.

FEEDING TRIAL 2

The results of the first feeding trial indicated the difficult nature of the task of carrying out successfully the paired feeding technique on animals fed the diets under discussion. Further, it had become obvious that although results such as had been obtained were of some comparative value when arrived at consistently in repeated experiments; nevertheless, the large variations observed in the values within groups made it extremely difficult to demonstrate any clear cut superiority of one protein food over another. The principal factor contributing to the large variation in biological values was found to be the wide range in initial weight. Accordingly rats weighing 50 ± 2.5 grams were used in the subsequent feeding trials. In view of the similarity in palatability of the different diets, it was decided

to let the animals eat ad libitum except when any rat started to consume markedly more than the others. In such cases the amount of food offered was lowered. Only one change, a trivial one to facilitate the preparation of the diets, was made in the basal diet; choline chloride was incorporated in the diet along with the other dry ingredients instead of being included in the vitamin mixture. Since Mitchell and Carman (1926) have shown that the protein content of new tissue is not always the same on different diets, the nitrogen retention method of McCollum and Shukers (cf. McCollum and Simmonds 1929) was utilized. A comparison was thus made possible between the two methods of determining biological values and in addition information was gained regarding the effects of the different diets on the composition of new tissue. Individual nitrogen determinations were performed on a control group of ten rats at the start and on each test rat at the end of the experiment. The intestines were stripped of their contents and the carcasses hydrolyzed with 8 to 10 N H_2SO_4 for 10 to 12 hours. The resulting hydrolysate was made up to volume and aliquots were then pipetted for macro-kjeldahl determinations. A statistical analysis of the percentage nitrogen compositions of the different groups revealed that there were no significant differences. Z value for $p = 0.05$ was 0.8639, found: 0.3724. The average values agreed within a narrow range (2.72 to 2.83%). It therefore appeared that under the conditions herein described, gain in body weight afforded a true indication of protein anabolism. Significant in this respect is the close parallelism of the values obtained by the nitrogen retention and growth methods (table III).

TABLE III. Biological values of fish and beef flesh proteins. Initial wt. of rats: 50 ± 2.5 g. Days on test diets: 28. Number of rats per group: 10. Basal diet: B (same as basal diet used in feeding trial no. 1 except that choline chloride was not included in the vitamin mixture but added along with the other dry ingredients).

Source of dietary protein	Daily food intake (g.)	Av. gain in wt. (g.)	Gains corr. for food intake (g.)	Gain in wt. per g. protein eaten	% Nitrogen retained of nitrogen ingested.
Lingcod	8.9	61.8	63.0	3.11	56.9
Halibut	9.1	64.4	63.6	3.15	57.7
Lemon sole	9.2	63.0	61.4	3.04	55.4
Salmon	8.9	62.5	63.1	3.12	55.0
Beef	8.9	56.3	56.9	2.80	50.6

A statistical analysis of the gains was carried out by the covariance method in which the variance of the actual gains, freed from the effects of varying food consumption by regression, was subjected to analysis. This procedure was carried out in preference to an analysis of the gains in weight per gram of protein eaten, because, as Crampton (1934) has pointed out, a statistical analysis of the latter ratios includes "in addition to the experimental error, a systematic error which may be of sufficient magnitude to seriously distort the result, and vitiate

the estimate of experimental error and hence any test of significance." The systematic error is of course owing to the fact that no allowance is made in the calculation of the biological values for the amount of food required for maintenance (Crampton and Hopkins 1934a, b). The differences in the corrected gains of the rats made on the fish and beef flesh diets were found to be not quite large enough to be termed significant. The necessary difference between means for P = 0.05 was 7.4 grams; the difference found between the average corrected gain on the beef ration and the highest average corrected gain recorded for the fish ration was 6.7 grams.

Two control diets were fed consisting in the case of diet E of a supplement of 20% vitamin-free casein to the basal diet and in the case of diet F of a supplement of 20% casein plus 5% yeast and 1% liver concentrate (cf. table I). The rats on the latter diet gained 103.4 grams and those on the former diet gained 96.5 grams. Much of this difference in gain may be attributed to difference in food intakes (9.6 g. and 9.8 g. per day on diets E and F respectively). A statistical analysis of the data revealed that the difference could not be termed significant. The necessary difference between the corrected means for P = 0.05, was 10.38 grams; found = 5.06 grams. The biological values calculated for the two diets yielded figures of 1.77 and 1.63 for diets E and F respectively. It therefore appeared that the protein seemed to be utilized just as efficiently, if not more so, by the rats which did not receive the additional B-vitamins in the form of yeast and liver concentrates. On the other hand the higher protein content of diet F would tend to lead to a slightly lower value than that derived from diet E (Mitchell 1924). Further evidence regarding the adequacy of the basal diet is presented in data from trial 3.

Feeding Trial 3

The previous experiment showed that in order to attain the precision necessary to distinguish differences in the biological values of the protein preparations under study, further modifications in experimental conditions had to be made. Initial weight and food intakes were comparable; consequently about the only other main source of variation was the inclusion of males and females in the same groups.

An examination of the results from trial 2 revealed that the values derived from the females were slightly but consistently lower than those obtained from the males despite the fact that the average food intake of the latter was only 249.5 grams as compared to 253.3 grams for the females. Accordingly feeding trial 3 was set up so that adequate numbers of each sex were placed on each diet. Since the values for the four fish flesh products were essentially identical, one (halibut) was chosen at random to be used in further comparative tests. In this way enough rats could be used on each diet to afford a fair test of the differences between groups of rats fed diets containing beef and the fish flesh proteins.

Two changes were made in the basal diet: 10% mazola oil was substituted for an equal amount of beef dripping, and 0.25% each of yeast and liver concentrates was added (see diet C, table I). The latter materials were added to deter-

mine whether or not the increased supply of B-vitamins would affect the efficiency of protein utilization. These concentrates effected the introduction of about 0.28% non-test protein into the diets, or about 3.5% of the total dietary protein. The results are shown in table IV, with additional values for lingcod, lemon sole, white spring salmon, herring, and red snapper, obtained about a year later. The rats were allowed to eat ad libitum.

The data from the animals fed the halibut and beef diets were subjected to the same type of statistical analysis utilized to assess the results of feeding trial 2. The necessary difference between the average gains for P = 0.01 was found to be

TABLE IV. Biological values of fish and beef flesh proteins. Initial wt. of rats: 50 ± 2.5 g. Days on test diets: 28. Basal diet: C.

Source of dietary protein	Sex	No. of rats	Daily food intake (g.)	Av. gain in wt. (g.)	Gain corr. for food intake (g.)	Gain in wt. per g. protein eaten
Halibut	M	20	10.3	75.5	74.9	3.27
Halibut	F	20	10.5	70.1	68.1	2.98
Beef	M	20	10.2	65.3	66.0	2.86
Beef	F	20	10.1	59.7	61.6	2.63
Lingcod	M	15	8.9	71.7	—	3.60
Lemon sole	M	15	8.9	72.8	—	3.66
White spring salmon	M	15	9.3	77.2	—	3.68
Red snapper	M	15	9.0	78.8	—	3.86
Herring	F	15	8.8	61.4	—	3.18

4.9 and 4.4 grams for the male and female groups respectively. The corresponding differences observed were 9.0 and 6.5 grams and these may therefore be termed highly significant differences.

The remarkably high values obtained for the last five preparations given in table IV are difficult to explain. The figures 3.60 to 3.86 for the males and 3.18 for the females at an 8% protein level are definitely higher than any value previously obtained. The only possible explanation of which the writer can think is that the health and vigour of the rat colony had improved markedly in the interval which had elapsed between the feeding experiments. (In this connection, it might be pointed out that the feeding and care of the rat colony, about one and a half years previously, had undergone some considerable change.) These values are the highest ever reported by this method on proteins of importance in human nutrition. As in trials 1 and 2, the data for lingcod, lemon sole, and white spring salmon agree closely. Such a finding emphasizes the desirability of performing comparative quantitative experiments at the same time or within short time intervals. The value found for the female rats on the herring diet is also higher than that previously found on the halibut and beef flesh diets, although it is in good agreement with the figure obtained from the females on the

diet containing 10% halibut flesh protein in trial 5 (see table VII) which was performed at about the same time.

It is noteworthy that the average biological values for the rats on the halibut (3.13) and beef (2.75) diets are almost exactly the same as those obtained on these diets in the previous experiment in which no yeast or liver concentrates were incorporated (3.15 and 2.80 respectively). Although, indeed, greater gains were observed on the former diets, these could be wholly attributed to greater food intakes as indicated by the similarity in biological values, the calculation of which involves a crude correction for differing food intakes. It would appear from these results that the inclusion of liver and yeast concentrates did not increase the efficiency of utilization of protein, and, further, that the five crystalline vitamins along with the factors present in the flesh and the elements synthesized in the gut on such diets permitted maximal utilization of protein (cf. Beveridge 1946).

Two control diets containing 20% casein as the protein moiety were fed to groups of 10 males each. One diet, G, contained only the amount of yeast and liver concentrates indicated in the test diets. The other diet, H, contained increased amounts of these vitamin supplements, 1% instead of 0.25% of each (see table I). The rats on H ration showed an average gain of 132.0 grams on a food intake of 11.3 grams per day; those on G ration showed an average gain of 112.7 grams on a food intake of 10.3 grams per day. In the latter case, the elimination of data from a rat which gave an abnormally poor growth response (a gain in weight of only 66.8 grams) would have raised the average gain to 117.7 grams. When these data are expressed in the form of gain in weight per gram of protein eaten, the value derived from the data of diet G is 2.14, whereas that from H is 1.98. This result constitutes further evidence that the additions of the yeast and liver concentrates, although increasing the rate of growth under these conditions, do not promote increased efficiency in protein anabolism. The evidence presented permits the conclusion that in the diets employed protein was the principal and almost certainly the only factor which limited protein anabolism.

Feeding Trial 4

Dr. A. T. Cameron, Chairman of the Fisheries Research Board of Canada, suggested that, before publication of these results, another feeding trial at a higher protein level be carried out. This suggestion was adopted and the experiment was performed exactly as in the preceding trial except that crude protein (total dietary N $\times$ 6.25) was incorporated at a level of 12%. A test diet containing 12% casein was also included.

Although the difference in the average gains of the male rats on the beef and halibut diets (table V) was shown to be statistically significant by means of the analysis already cited, the difference in gains between the female rats was a little too small to be judged significant. The necessary difference between the corrected average gains of the males for $P = 0.05$ was 4.9 grams, found: 6.3 grams; the

corresponding data found for the females for P = 0.05, necessary difference: 4.9 grams, found: 4.1 grams.

TABLE V. Biological values of halibut and beef flesh proteins. Initial wt. of rats: 50 ± 2.5 g. Days on test diets: 28. Basal diet: D.

Source of dietary protein	Sex	No. of rats	Daily food intake (g.)	Av. gain in wt. (g.)	Gains corr. for food intake (g.)	Gain in wt. per g. protein eaten
Halibut	M	17	12.4	138.3	135.7	3.32
Halibut	F	16	10.5	95.6	95.7	2.66
Beef	M	17	12.0	126.9	129.4	3.15
Beef	F	16	10.6	91.7	91.6	2.58

The fact that the difference in average gains between the females on the beef and halibut diets could not be shown to be significant was not surprising, since the level at which the test proteins were fed approached and perhaps equalled the level at which maximal growth is elicited. In this connection it may be recalled that the average gain made by the animals on the adequate control diet H was 132.0 grams, and that brought about by the halibut diet was 138.3 grams. Food intakes were 11.3 and 12.4 grams respectively. Obviously under such conditions the protein content is not a factor limiting growth and further, as both Osborne and Mendel (1916) and Mitchell (1924) have pointed out, the possibility that an excess of protein above the requirements for maintenance and growth has been consumed, renders any ratio obtained of doubtful value as a measure of the biological adequacy of the particular protein under investigation.

The gains in the six male rats fed the casein diet were compared with those resulting from the corresponding animals on the beef and halibut diets (table VI). Application of the type of statistical analysis to which reference has already been made revealed that for probabilities of 0.05 and 0.01 the differences in the corrected average gains should be 8.1 and 12.3 grams respectively. The difference in the corrected average gains effected by the casein and beef diets was 11.2 grams in favour of the latter ration and may be termed significant; the corre-

TABLE VI. Biological values of casein and flesh proteins of beef and halibut. Initial wt. of rats: 50 ± 2.5 g. Days on test diets: 28. Basal diet: D.

Source of dietary protein	No. of rats	Av. daily food intake (g.)	Av. gain in wt. (g.)	Gains corr. for food intake (g.)	Gain in wt. per g. protein eaten
Halibut	6	12.3	136.1	130.7	3.30
Beef	6	12.2	129.7	126.2	3.19
Casein	6	10.8	104.8	115.0	2.89

sponding figure for the casein and halibut diets was 15.7 grams in favour of the fish flesh diet and may be termed highly significant. It therefore appears that casein has a biological value definitely below those of halibut and beef flesh proteins.

EFFECT OF SEX

In a series of experiments to determine the nutritive value of crude protein in animal parts, Hoagland and Snider (1926b) presented evidence showing that larger biological values were obtained from male than from female rats. The

TABLE VII. Comparison of biological values derived from male and female rats.

Feeding trial	Diets	No. of rats	Sex	Protein level (%)	Av. food intake (g.)	Gain in wt. per g. protein eaten
2.		20	M	8	249.5	3.17
		30	F	8	253.3	2.95
3.	Halibut	20	M	8	289.1	3.27
	Halibut	20	F	8	293.6	2.98
	Beef	20	M	8	285.3	2.86
	Beef	20	F	8	283.1	2.63
5.	Halibut	19	M	10	291.0	3.29
	Halibut	19	F	10	291.0	3.13
4.	Halibut	17	M	12	346.6	3.32
	Halibut	16	F	12	295.1	2.66
	Beef	17	M	12	335.3	3.15
	Beef	16	F	12	295.7	2.58

work of these authors is inconclusive since only a small number of each sex was put on a diet. In many cases the "groups" consisted of only one or two rats. Of fifty comparisons reported, however, all except two showed relatively large margins in favour of the males. The remaining two were about equal. This consistency is all the more remarkable because of the small number of animals utilized in each group. Morgan (1931), on the other hand, has published data from which she concluded that the values derived from the two sexes were essentially the same. An examination of the values cited revealed that of six comparisons, the biological values derived from the males were higher, although only by quite small margins, in five cases; in the remaining instance, the value derived from the female was slightly higher. Morgan's work is inconclusive since only small numbers of each sex were retained on some of the diets.

The results obtained from the males and females of similar initial weights in the present study (trials 2 to 5) are shown in table VII. Despite the fact that the food intakes of the males and females were almost exactly the same in

trials 2 and 3, the biological values derived from the former animals were higher in every case. In trial 4 the males ate significantly more than did the females and the margins by which the values obtained from the males exceeded those from the females was much higher than in the previous trials. It may be of interest to note the consistency in the differences in biological values derived from the two sexes at similar protein levels. For example in trial 2 the difference was 0.22, in trial 3 on the halibut diet, 0.29, and on the beef diet, 0.23. At the 12% protein level in trial 4 the differences were not quite so consistent being 0.66 and 0.57 for the halibut and beef flesh diets respectively.

The difference between the corrected average gains of males and females was in every case shown to be highly significant. For example the differences between the corrected means of the gains made by the male and female rats on the beef diets were, at the 8% protein level, 4.91 grams, and at the 12% protein level, 22.41 grams. For P = 0.01 the necessary differences were found to be 3.96 and 8.85 grams respectively.

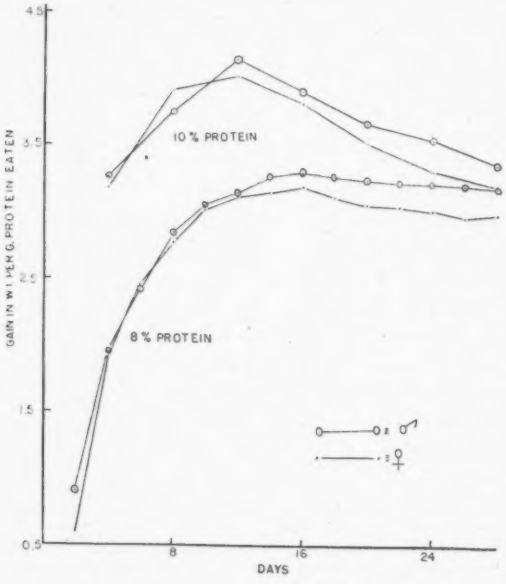
These findings appeared to indicate that the male rats actually utilized the protein of their ration more efficiently for growth. It was felt that such an unexpected result warranted fuller investigation and, in the course of some later work in determining the biological value of certain proteins, a group of 19 males and females were paired-fed on a diet containing halibut flesh protein at a level of 10%. The animals were very carefully paired, the initial weights of the paired rats being within 0.5 grams of each other and usually within 0.2 grams. The test diet was the same as diet C (table I), except that the test protein was incorporated at the level just now indicated. The paired feeding was highly successful. Average gains of 95.8 grams and 91.1 grams were recorded for the males and females respectively. The necessary difference for P = 0.05 was found to be 3.50 grams and for P = 0.01, 4.77 grams. The observed difference, 4.70 grams, therefore just fails to be of sufficient magnitude to be judged highly significant but may be termed significant. The pertinent question as to whether or not the composition of the new tissue of the males and females differed might very well be raised at this point. Data regarding this are available from feeding trial 2. Although the average percentage nitrogen composition differed by only 0.005% (2.793% and 2.788%), and by inspection seemed not significantly different, an analysis of variance was performed on the series. The necessary difference for P = 0.05 was found to be 0.226. The observed difference was therefore definitely not significant. Furthermore, an examination of the data obtained by the nitrogen retention method of McCollum and Shukers (cf. McCollum and Simmonds 1929) in trial 2 revealed that the average biological value derived from the males was 54.9, and that from the females, 51.1. This result substantiates the evidence previously presented that male rats, under the conditions herein described, utilize the dietary protein more efficiently for growth than do the females.

In figure 1, shown in the section concerned with the effect of length of feeding time on biological value, it is apparent that the efficiency of utilization of protein in the female rats does not fall below that of the males until about half way

through in the 28-day test period. This phenomenon roughly corresponds with the marked flattening of the growth curve of the females and it would appear that the food intake of the female does not decrease correspondingly with decrease in growth rate. (The somewhat higher value obtained on the eighth day from the females on the diet containing 10% protein is due to a slightly higher food consumption during the first few days than was evidenced by the males).

The differences in food intake and biological values observed in trial 4 are typical of the work and confirm the findings reported by Hoagland and Snider (1926 a, b). The latter attributed their result to the fact that, since the male

FIGURE 1. Relationship between length of time of feeding and efficiency of protein utilization by male and female rats.



rats ate more, they required a smaller proportion of their food intake for maintenance than did the females. On the other hand, as Morgan (1931) has pointed out, a neutralizing tendency would be the increasing maintenance requirements of the larger animals.

The steeper growth curve of the males is a well known phenomenon and any change which brings about conditions more nearly optimal for growth will permit the male rats to attain a growth rate more nearly normal and accentuate the difference in values shown by the two sexes, (compare results on the 8 and 12% protein diets, table VII). Paired feeding naturally will depress the curve and consequently differences between biological values derived from males and females will be correspondingly more difficult to discern since the potentiality for growth of the female will not have been altered to the same extent as that

for the male. The same holds true when proteins of low biological value are fed to the two sexes as Morgan (1931) did. It is, therefore, not surprising that the latter did not observe marked differences in the ratios obtained for the two sexes. The flatter growth curve of female rats is fairly evident in the period shortly after weaning and, since the evidence presented here indicates that the dietary protein is not so efficiently utilized as by male rats, it would appear that the food intake of the female does not decrease to the same extent as does the growth rate. A number of possible explanations for the sex difference in protein utilization may be mentioned: (1) a greater proportion of protein may be catabolized by the females; (2) males may digest and absorb a slightly larger percentage of ingested protein; (3) the proportion of essential amino acids required to promote maximal growth in females may differ from that required for males.

EFFECT OF LENGTH OF TEST PERIOD

In figure 1 is shown the relationship between the calculated biological values and times for the males and females of feeding trials 2 and 5. Boas-Fixsen (1935) published a review in which she asserted that the miminal length of feeding time required for accuracy was 60 days. The data presented in figure 1 indicate that in about 16 days the protein efficiency ratios reach a peak and then assume fairly constant values which, of course, diminish gradually with time. The value for the males on the 8% protein diet decreased from a peak of 3.31 at 16 days to 3.21 at 28 days. The corresponding values for the females were 3.20 and 3.00. The curve obtained from the animals on the 10% protein diet differs to some extent from that obtained from the animals fed the diet containing the lower level of protein. It may be worth while to recall that the animals on the former diet were paired-fed although some slight divergence in food intake occurred during the first few days. The latter diet was fed as previously described on a slightly restricted basis. It may be concluded that the results obtained in this investigation indicate that an experiment of 60 days' duration is unnecessarily long and wasteful of time and material; whereas 21 to 28 days' feeding time appears to be adequate in determining the relative effectiveness of proteins for growth.

DISCUSSION

Because of the many variables involved in the determination of the biological value of proteins it is difficult to compare adequately results obtained from other laboratories. Lanham and Lemon (1938) have reported values for a number of fish flesh preparations only one of which was treated in the present investigation. The values found by them varied from 1.88 to 2.23. Their preparations, however, were made in a different way (acetone extraction); protein level utilized was 9%; and the feeding period was 10 weeks. It is interesting to note, however, that these workers also found that the crude proteins of fish flesh were definitely superior to those of beef flesh. In an extensive study on the nutritive value of crude protein in animal parts, Hoagland and Snider (1926b) obtained values for beef muscle of 3.15 for male rats and 3.00 for females. The initial weight of the

rats was about 40 grams, protein level (N × 6.25) 10%, and feeding period 30 days. These conditions are not exactly the same as, but approximate those employed here. The biological values, considering the somewhat different conditions, agree fairly well. The same workers, in an earlier paper (1926a) reported a value of 1.98 for casein obtained under similar conditions with male rats. The value of 2.89 found in the present work, even when the differing conditions are taken into account, is widely divergent. Hoagland and Snider did not demonstrate the adequacy of their basal diet, and the author suggests that the foregoing data indicate that it was inadequate. The fact that figures of the same order of magnitude were obtained at both laboratories on beef muscle may be explained by the probable presence in the beef of the factors absent or deficient in the basal diet. The casein tested by Hoagland and Snider was a "highly purified product" and consequently would contain negligible amounts of the B-vitamins; this preparation would not, therefore, make up any deficiency in the basal diet. Under such circumstances the food intake would be low and a low biological value would be obtained. Both these features exist in the report under consideration. This situation on the other hand would also happen when a protein of low biological value was being tested. However, the result of the present study indicates that casein is a protein of relatively high biological value and hence the adequacy of the basal diet used by Hoagland and Snider must be questioned.

SUMMARY

1. The biological values of the crude flesh proteins of lingcod, halibut, lemon sole, white spring salmon, red snapper, herring, beef and of egg albumin have been determined using rats of initial weight 50 ± 2½ grams, a feeding period of 28 days, and a protein level of 8%.

2. The gains brought about by the fish flesh proteins corrected for varying food intake were shown to be significantly greater than those brought about by the beef flesh proteins. The biological value of egg albumin was of the same order of magnitude as that of the beef flesh proteins.

3. Under the same conditions listed in point (1) but at a protein level of 12%, the biological values of the proteins of halibut flesh, beef flesh, and casein were compared. The biological values of the two flesh proteins were shown to be significantly greater than that of casein, and that of halibut significantly greater than the value derived from the beef flesh proteins.

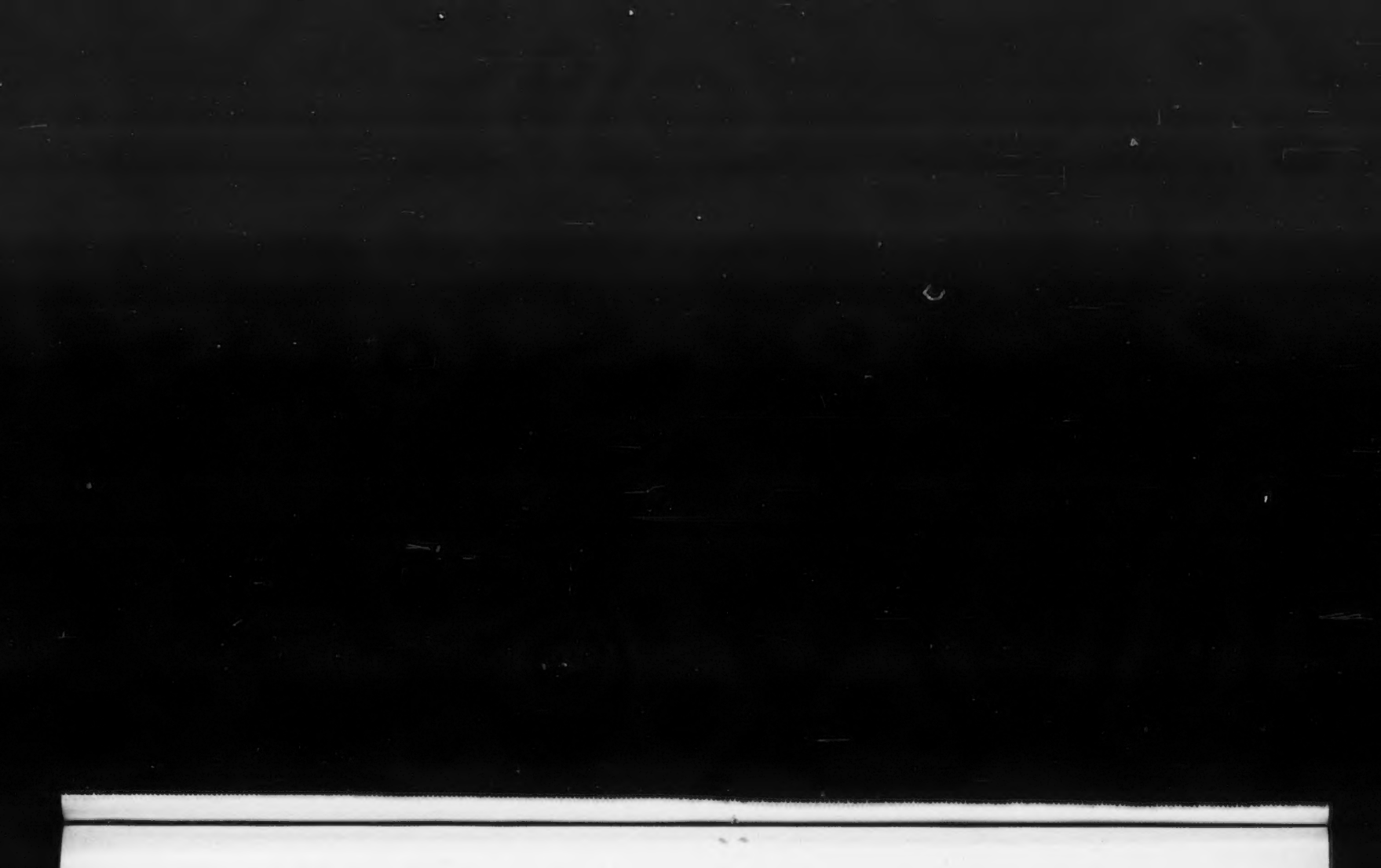
4. Evidence has been submitted to show that male rats even at similar food intakes utilize the protein more efficiently for growth than do females.

5. The graph of efficiency of protein utilization against time revealed that feeding periods of 21 to 28 days are entirely adequate in determining the biological value of proteins.

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the help of Mr. V. Grigg of the B. C. Industrial and Scientific Research Council in guiding the author through the application of the statistical method utilized in assessing the results. Thanks are also due

to Messrs. O. C. Young and E. P. Sidaway for their assistance in cooking and drying the first of the flesh preparations, and to Misses Phyllis Boyce and Lucille Gardner for the feeding and care of the experimental animals and to the latter for performance of some of the technical work.

REFERENCES

BEVERIDGE, J. M. R. *J. Fish. Res. Bd. Can.*, **7** (2) 1947.
BEVERIDGE, J. M. R., AND C. C. LUCAS. *J. Biol. Chem.*, **157**, 311-321, 1945.
BOAS-FIXSEN, M. A. *Nutr. Abstr. Rev.*, **4**, 447-459, 1935.
CANNON, P. R., W. E. CHASE AND R. W. WISSLER. *J. Immunol.*, **47**, 133-417, 1943.
CANNON, P. R., R. W. WISSLER, R. L. WOOLRIDGE AND E. P. BENDITT. *Ann. Surg.*, **120**, 514-525, 1944.
CO TUI. *J. Amer. Diet. Assn.*, **22**, 97-109, 1946.
CRAMPTON, E. W. *J. Nutr.*, **7**, 305-320, 1934.
CRAMPTON, E. W., AND J. W. HOPKINS. *J. Nutr.*, **8**, 113-123, 1934 a.
J. Nutr., **8**, 329-346, 1934 b.
ELMAN, R. *Physiol. Rev.*, **24**, 372-389, 1944.
HOAGLAND, R., AND G. G. SNIDER. *J. Agri. Res.*, **32**, 679-688, 1926 a.
J. Agri. Res., **32**, 1025-1040, 1926 b.
LANHAM, W. B., AND J. M. LEMON. *Food Res.*, **3**, 549-553, 1938.
MCCOLLUM, E. V., AND N. SIMMONDS. The newer knowledge of nutrition. 4th Ed., The MacMillan Co., New York, 1-594, 1929.
MILLER, L. L., J. F. ROSS AND G. H. WHIPPLE. *Amer. J. Med. Sci.*, **200**, 739-756, 1940.
MITCHELL, H. H. *Physiol. Rev.*, **4**, 424-478, 1924.
MITCHELL, H. H., AND G. G. CARMAN. *Amer. J. Physiol.*, **76**, 398-410, 1926.
MORGAN, A. F. *J. Biol. Chem.*, **90**, 771-792, 1931.
MORRIS, H. P., C. S. DUBNIK AND T. B. DUNN. *J. Nat. Cancer Inst.*, **5**, 271-282, 1945.
OSBORNE, T. B., AND L. B. MENDEL. *J. Biol. Chem.*, **26**, 1-23, 1916.
OSBORNE, T.B., L. B. MENDEL, AND E. L. FERRY. *J. Biol. Chem.*, **37**, 223-229, 1919.
STARE, F. J., D. M. HEGSTED AND J. M. MCKIBBIN. *Ann. Rev. Biochem.*, **14**, 431-468, 1945.
THOMPSON, W.D., I. S. RAVDIN AND J. E. RHOADS. *Arch. Surg.*, **36**, 500-508, 1938.